

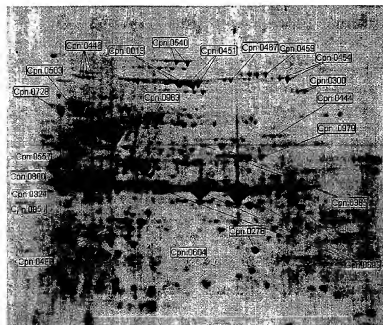
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(54) Title: IMMUNISATION AGAINST *CHLAMYDIA PNEUMONIAE*

(57) Abstract: The published genomic of *Chlamydia pneumoniae* reveals over 1000 putative encoded proteins but does not itself indicate which of these might be useful antigens for immunisation and vaccination or for diagnosis. This difficulty is addressed by the invention, which provides a number of *C. pneumoniae* protein sequences suitable for vaccine production and development and/or for diagnostic purposes.



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## IMMUNISATION AGAINST *CHLAMYDIA PNEUMONIAE*

All documents cited herein are incorporated by reference in their entirety.

### TECHNICAL FIELD

This invention is in the field of immunisation against chlamydial infection, in particular against  
5 infection by *Chlamydia pneumoniae*.

### BACKGROUND ART

*Chlamydiae* are obligate intracellular parasites of eukaryotic cells which are responsible for endemic sexually transmitted infections and various other disease syndromes. They occupy an exclusive eubacterial phylogenetic branch, having no close relationship to any other known organisms – they are  
10 classified in their own order (*Chlamydiales*) which contains a single family (*Chlamydiaceae*) which in turn contains a single genus (*Chlamydia*). A particular characteristic of the *Chlamydiae* is their unique life cycle, in which the bacterium alternates between two morphologically distinct forms: an extracellular infective form (elementary bodies, EB) and an intracellular non-infective form (reticulate bodies, RB). The life cycle is completed with the re-organization of RB into EB, which  
15 subsequently leave the disrupted host cell ready to infect further cells.

Four chlamydial species are currently known – *C.trachomatis*, *C.pneumoniae*, *C.pecorum* and *C.psittaci* [e.g. Raulston (1995) *Mol Microbiol* 15:607-616; Everett (2000) *Vet Microbiol* 75:109-126]. *C.pneumoniae* is closely related to *C.trachomatis*, as the whole genome comparison of at least two isolates from each species has shown [Kalman *et al.* (1999) *Nature Genetics* 21:385-389; Read  
20 *et al.* (2000) *Nucleic Acids Res* 28:1397-406; Stephens *et al.* (1998) *Science* 282:754-759]. Based on surface reaction with patient immune sera, the current view is that only one serotype of *C.pneumoniae* exists world-wide.

*C.pneumoniae* is a common cause of human respiratory disease. It was first isolated from the conjunctiva of a child in Taiwan in 1965, and was established as a major respiratory pathogen in  
25 1983. In the USA, *C.pneumoniae* causes approximately 10% of community-acquired pneumonia and 5% of pharyngitis, bronchitis, and sinusitis.

More recently, the spectrum of *C.pneumoniae* infections has been extended to include atherosclerosis, coronary heart disease, carotid artery stenosis, myocardial infarction, cerebrovascular disease, aortic aneurysm, claudication, and stroke. The association of *C.pneumoniae* with  
30 atherosclerosis is corroborated by the presence of the organism in atherosclerotic lesions throughout the arterial tree and the near absence of the organism in healthy arterial tissue. *C.pneumoniae* has also been isolated from coronary and carotid atheromatous plaques. The bacterium has also been associated with other acute and chronic respiratory diseases (e.g. otitis media, chronic obstructive pulmonary disease, pulmonary exacerbation of cystic fibrosis) as a result of sero-epidemiologic  
35 observations, case reports, isolation or direct detection of the organism in specimens, and successful

response to anti-chlamydial antibiotics. To determine whether chronic infection plays a role in initiation or progression of disease, intervention studies in humans have been initiated, and animal models of *C.pneumoniae* infection have been developed.

Considerable knowledge of the epidemiology of *C.pneumoniae* infection has been derived from serologic studies using the *C.pneumoniae*-specific microimmunofluorescence test. Infection is ubiquitous, and it is estimated that virtually everyone is infected at some point in life, with common re-infection. Antibodies against *C.pneumoniae* are rare in children under the age of 5, except in developing and tropical countries. Antibody prevalence increases rapidly at ages 5 to 14, reaching 50% at the age of 20, and continuing to increase slowly to ~80% by age 70.

A current hypothesis is that *C.pneumoniae* can persist in an asymptomatic low-grade infection in very large sections of the human population. When this condition occurs, it is believed that the presence of *C.pneumoniae*, and/or the effects of the host reaction to the bacterium, can cause or help progress of cardiovascular illness.

It is not yet clear whether *C.pneumoniae* is actually a causative agent of cardiovascular disease, or whether it is just artefactually associated with it. It has been shown, however, that *C.pneumoniae* infection can induce LDL oxidation by human monocytes [Kalayoglu *et al.* (1999) *J. Infect. Dis.* 180:780-90; Kalayoglu *et al.* (1999) *Am. Heart J.* 138:S488-490]. As LDL oxidation products are highly atherogenic, this observation provides a possible mechanism whereby *C.pneumoniae* may cause atheromatous degeneration. If a causative effect is confirmed, vaccination (prophylactic and therapeutic) will be universally recommended.

Genomic sequence information has been published for *C.pneumoniae* [Kalman *et al.* (1999) *supra*; Read *et al.* (2000) *supra*; Shirai *et al.* (2000) *J. Infect. Dis.* 181(Suppl 3):S524-S527; WO99/27105; WO00/27994] and is available from GenBank. Sequencing efforts have not, however, focused on vaccination, and the availability of genomic sequence does not in itself indicate which of the >1000 genes might encode useful antigens for immunisation and vaccination. WO99/27105, for instance, implies that every one of the 1296 ORFs identified in the *C.pneumoniae* strain CM1 genome is a useful vaccine antigen.

It is thus an object of the present invention to identify antigens useful for vaccine production and development from amongst the many proteins present in *C.pneumoniae*. It is a further object to identify antigens useful for diagnosis (*e.g.* immunodiagnosis) of *C.pneumoniae*.

## DISCLOSURE OF THE INVENTION

The invention provides proteins comprising the *C.pneumoniae* amino acid sequences disclosed in the examples.

It also provides proteins comprising sequences which share at least x% sequence identity with the *C.pneumoniae* amino acid sequences disclosed in the examples. Depending on the particular



sequence,  $x$  is preferably 50% or more (e.g. 60%, 70%, 80%, 90%, 95%, 99% or more). These include mutants and allelic variants. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence. Identity between proteins is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty*=12 and *gap extension penalty*=1.

The invention further provides proteins comprising fragments of the *C.pneumoniae* amino acid sequences disclosed in the examples. The fragments should comprise at least  $n$  consecutive amino acids from the sequences and, depending on the particular sequence,  $n$  is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 30, 40, 50, 75, 100 or more). Preferably the fragments comprise one or more epitope(s) from the sequence. Other preferred fragments omit a signal peptide.

The proteins of the invention can, of course, be prepared by various means (e.g. native expression, recombinant expression, purification from cell culture, chemical synthesis etc.) and in various forms (e.g. native, fusions etc.). They are preferably prepared in substantially pure form (i.e. substantially free from other *C.pneumoniae* or host cell proteins). Heterologous expression in *E.coli* is a preferred preparative route.

According to a further aspect, the invention provides nucleic acid comprising the *C.pneumoniae* nucleotide sequences disclosed in the examples. In addition, the invention provides nucleic acid comprising sequences which share at least  $x\%$  sequence identity with the *C.pneumoniae* nucleotide sequences disclosed in the examples. Depending on the particular sequence,  $x$  is preferably 50% or more (e.g. 60%, 70%, 80%, 90%, 95%, 99% or more).

Furthermore, the invention provides nucleic acid which can hybridise to the *C.pneumoniae* nucleic acid disclosed in the examples, preferably under "high stringency" conditions (e.g. 65°C in a 0.1xSSC, 0.5% SDS solution).

Nucleic acid comprising fragments of these sequences are also provided. These should comprise at least  $n$  consecutive nucleotides from the *C.pneumoniae* sequences and, depending on the particular sequence,  $n$  is 10 or more (e.g. 12, 14, 15, 18, 20, 25, 30, 35, 40, 50, 75, 100, 200, 300 or more).

According to a further aspect, the invention provides nucleic acid encoding the proteins and protein fragments of the invention.

It should also be appreciated that the invention provides nucleic acid comprising sequences complementary to those described above (e.g. for antisense or probing purposes).

Nucleic acid according to the invention can, of course, be prepared in many ways (e.g. by chemical synthesis, from genomic or cDNA libraries, from the organism itself etc.) and can take various forms (e.g. single stranded, double stranded, vectors, probes etc.).

In addition, the term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) *etc.*

According to a further aspect, the invention provides vectors comprising nucleotide sequences of the invention (*e.g.* cloning or expression vectors) and host cells transformed therewith.

- 5 According to a further aspect, the invention provides immunogenic compositions comprising protein and/or nucleic acid according to the invention. These compositions are suitable for immunisation and vaccination purposes. Vaccines of the invention may be prophylactic or therapeutic, and will typically comprise an antigen which can induce antibodies capable of inhibiting (a) chlamydial adhesion, (b) chlamydial entry, and/or (c) successful replication within the host cell. The vaccines  
10 preferably induce any cell-mediated T-cell responses which are necessary for chlamydial clearance from the host.

The invention also provides nucleic acid or protein according to the invention for use as medicaments (*e.g.* as vaccines). It also provides the use of nucleic acid or protein according to the invention in the manufacture of a medicament (*e.g.* a vaccine or an immunogenic composition) for  
15 treating or preventing infection due to *C.pneumoniae*.

The invention also provides a method of treating (*e.g.* immunising) a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid or protein according to the invention.

According to further aspects, the invention provides various processes.

- 20 A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression.

A process for producing protein or nucleic acid of the invention is provided, wherein the protein or nucleic acid is synthesised in part or in whole using chemical means.

- A process for detecting *C.pneumoniae* in a sample is provided, wherein the sample is contacted with  
25 an antibody which binds to a protein of the invention.

A summary of standard techniques and procedures which may be employed in order to perform the invention (*e.g.* to utilise the disclosed sequences for immunisation) follows. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

#### General

- 30 The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature *e.g.* Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989) and *Third Edition* (2001); *DNA Cloning, Volumes I and II* (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed, 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I.  
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- Freshney ed. 1986); *Immunized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C. C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

#### Definitions

- 10 A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term "comprising" means "including" as well as "consisting" e.g. a composition "comprising" X may consist exclusively of X or may include something additional to X, such as X+Y.

- 15 The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Chlamydial sequence is heterologous to a mouse host cell. A further examples would be two epitopes from the same or different proteins which have been  
20 assembled in a single protein in an arrangement not found in nature.

- An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be  
25 reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

- 30 A "mutant" sequence is defined as DNA, RNA or amino acid sequence differing from but having sequence identity with the native or disclosed sequence. Depending on the particular sequence, the degree of sequence identity between the native or disclosed sequence and the mutant sequence is preferably greater than 50% (e.g. 60%, 70%, 80%, 90%, 95%, 99% or more, calculated using the Smith-Waterman algorithm as described above). As used herein, an "allelic variant" of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs essentially at the same locus in the genome of another or second isolate, and that, due to natural variation caused by, for example, mutation or recombination,  
35 has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5' or 3' untranslated regions of the gene, such as in regulatory control regions (e.g. see US patent 5,753,235).

### Expression systems

The Chlamydial nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, baculoviruses, plants, bacteria, and yeast.

#### i. Mammalian Systems

- 5 Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA  
10 synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation [Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A Laboratory Manual*, 2nd ed.].

- Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes  
15 simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallothionein gene, also provide useful promoter sequences. Expression may be either constitutive or regulated (inducible), depending on the promoter can be induced with glucocorticoid in hormone-responsive  
20 cells.

- The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription  
25 initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter [Maniatis et al. (1987) *Science* 236:1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.]. Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host range. Examples include the SV40 early gene enhancer [Dijkema et al. (1985) *EMBO J.* 4:761] and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus [Gorman et al.  
30 (1982) *PNAS USA* 79:6777] and from human cytomegalovirus [Boshart et al. (1985) *Cell* 41:521]. Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion [Sassone-Corsi and Borelli (1986) *Trends Genet.* 2:215; Maniatis et al. (1987) *Science* 236:1237].

- A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein  
35 will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader

fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus tripartite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

- 5 Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation [Birnstiel et al. (1985) *Cell* 41:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot
- 10 (1989) *Trends Biochem. Sci.* 14:105]. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator/polyadenylation signals include those derived from SV40 [Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*].

- Usually, the above described components, comprising a promoter, polyadenylation signal, and transcription
- 15 termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g. plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing
- 20 the replication systems of papovaviruses, such as SV40 [Gluzman (1981) *Cell* 23:175] or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replication systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 [Kaufman et al. (1989) *Mol. Cell. Biol.* 9:946] and pHEBO [Shimizu et al. (1986) *Mol. Cell. Biol.* 6:1074].

- The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion,
- 30 electroporation, encapsulation of polynucleotide(s) in liposomes, direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g. Hep G2), and a number of other cell lines.

## 35 ii. Baculovirus Systems

- The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site
- 40 for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence

homologous to the baculovirus-specific fragment in the transfer vector (this allows for the homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its own set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (*e.g.* plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) 17:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, 42:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (*e.g.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedrin protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlak et al., (1988), *J. Gen. Virol.* 69:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, 73:409). Alternatively, since the signals

for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human  $\alpha$ -interferon, Maeda et al., (1985), *Nature* 315:592; human gastrin-releasing peptide, Lebacqz-Verheyden et al., (1988), *Molec. Cell. Biol.* 8:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, 82:8404; mouse IL-3, (Miyajima et al., (1987) *Gene* 58:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, 7:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) 3:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays* 4:91. The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between ~1% and ~5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15  $\mu$ m in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus)

or absence (indicative of recombinant virus) of occlusion bodies. "Current Protocols in Microbiology" Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers & Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (WO 89/046699; Carbonell et al., (1985) *J. Virol.* 56:153; Wright (1986) *Nature* 321:718; Smith et al., (1983) *Mol. Cell. Biol.* 3:2156; and see generally, Fraser, et al. (1989) *In Vitro Cell. Dev. Biol.* 25:225).

Cells and cell culture media are commercially available for both direct and fusion expression of heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. See, e.g. Summers and Smith *supra*.

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, e.g. HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, e.g. proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

### iii. Plant Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as: US 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaulcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, *J. Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., *Nucleic Acids Research* 15:2515-2535 (1987); Wiersel et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, Gibberellins: in: *Advanced Plant Physiology*, Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)



Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker; and, for *Agrobacterium* transformations, T DNA sequences for *Agrobacterium*-mediated transfer to plant chromosomes. Where the heterologous gene is not readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilmink and Dons, 1993, *Plant Mol. Biol. Repr.*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's spliceosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet.*, 202:179-185, 1985. The genetic material may also be

transferred into the plant cell by using polyethylene glycol, Krens, et al., *Nature*, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl. Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersion*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, and *Datura*.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

#### iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in *Escherichia coli* (*E. coli*) [Raibaud *et al.* (1984) *Annu. Rev. Genet.* 18:173]. Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) [Chang *et al.* (1977) *Nature* 198:1056], and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) [Goeddel *et al.* (1980) *Nuc. Acids Res.* 8:4057; Yelverton *et al.* (1981) *Nucl. Acids Res.* 9:731; US patent 4,738,921; EP-A-0036776 and EP-A-0121775]. The *g*-lactamase (*bla*) promoter system [Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)], bacteriophage lambda PL [Shimatake *et al.* (1981) *Nature* 292:128] and T5 [US patent 4,689,406] promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter [US patent 4,551,433]. For example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor [Amann *et al.* (1983) *Gene* 25:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci.* 80:21]. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system [Studier *et al.* (1986) *J. Mol. Biol.* 189:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci.* 82:1074]. In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO-A-0 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon [Shine *et al.* (1975) *Nature* 254:34]. The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' and of *E. coli* 16S rRNA [Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological*

*Regulation and Development: Gene Expression* (ed. R.F. Goldberger)]. To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site [Sambrook *et al.* (1989) "Expression of cloned genes in *Escherichia coli*." In *Molecular Cloning: A Laboratory Manual*].

5 A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* or *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO-A-0 219 237).

10 Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene [Nagai *et al.* (1984) *Nature* 309:810]. Fusion proteins can also be  
15 made with sequences from the *lacZ* [Jia *et al.* (1987) *Gene* 60:197], *trpE* [Allen *et al.* (1987) *J. Biotechnol.* 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol.* 135:11], and *Chey* [EP-A-0 324 647] genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*e.g.* ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign  
20 protein. Through this method, native foreign protein can be isolated [Miller *et al.* (1989) *BioTechnology* 7:698].

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria [US patent 4,336,336]. The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is  
25 either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) [Masui *et al.* (1983), in: *Experimental Manipulation of Gene Expression*; Gharyeb *et al.* (1984) *EMBO J.* 3:2437] and the *E. coli* alkaline phosphatase signal sequence (*phoA*) [Oka *et al.* (1985) *Proc. Natl. Acad. Sci.* 82:7212]. As an additional example, the signal sequence of the alpha-amylase gene from various *Bacillus* strains can be used to secrete heterologous proteins from *B. subtilis* [Palva  
30 *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EP-A-0 244 042].

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g. plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various *Bacillus* strains integrate into the *Bacillus* chromosome (EP-A-0 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline [Davies *et al.* (1978) *Annu. Rev. Microbiol.* 32:469]. Selectable markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: *Bacillus subtilis* [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541], *Escherichia coli* [Shimatake *et al.* (1981) *Nature* 292:128; Amann *et al.* (1985) *Gene* 40:183; Studier *et al.* (1986) *J. Mol. Biol.* 189:113; EP-A-0 036 776, EP-A-0 136 829 and EP-A-0 136 907], *Streptococcus cremoris* [Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655]; *Streptococcus lividans* [Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655]; *Streptomyces lividans* [US patent 4,745,056].

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with  $\text{CaCl}_2$  or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. See e.g. [Masson *et al.* (1989) *FEMS Microbiol. Lett.* 60:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541, *Bacillus*], [Miller *et al.* (1988) *Proc. Natl. Acad. Sci.* 85:856; Wang *et al.* (1990) *J. Bacteriol.* 172:949, *Campylobacter*], [Cohen *et al.* (1973) *Proc. Natl. Acad. Sci.* 69:2110; Dower *et al.* (1988) *Nucleic Acids Res.* 16:6127; Kushner (1978) "An improved method for transformation of *Escherichia coli* with ColEI-derived plasmids. In *Genetic*

- Engineering: *Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol.* 53:159; Taketo (1988) *Biochim. Biophys. Acta* 949:318; Escherichia], [Chassy *et al.* (1987) *FEMS Microbiol. Lett.* 44:173 Lactobacillus]; [Fiedler *et al.* (1988) *Anal. Biochem* 170:38, Pseudomonas]; [Augustin *et al.* (1990) *FEMS Microbiol. Lett.* 66:203, Staphylococcus],
- 5 [Barany *et al.* (1980) *J. Bacteriol.* 144:698; Harlander (1987) \*Transformation of Streptococcus lactis by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun.* 32:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655; Somkuti *et al.* (1987) *Proc. 4th Eur. Cong. Biotechnology* 1:412, Streptococcus].

#### v. Yeast Expression

- 10 Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may
- 15 also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

- Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EP-A-0 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO-A-0 329 203). The yeast *PHO5* gene, encoding acid phosphatase, also provides useful promoter sequences [Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:1].
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- 25 In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (US Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of either the *ADH2*, *GAL4*, *GAL10*, OR *PHO5* genes, combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EP-A-0 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia*, [Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA* 77:1078; Henikoff *et al.* (1981) *Nature* 283:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol.* 96:119; Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast *Saccharomyces cerevisiae*," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene* 11:163; Panthier *et al.* (1980) *Curr. Genet.* 2:109:].
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A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always

be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See *e.g.* EP-A-0 196 056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*e.g.* ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (*e.g.* WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the genes for invertase (EP-A-0012873; JPO 62,096,086) and A-factor (US patent 4,588,684). Alternatively, leaders of non-yeast origin exist, such as an interferon leader, that also provide for secretion in yeast (EP-A-0060057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (US Patents 4,546,083 and 4,870,008; EP-A-0 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alphafactor. (*e.g.* see WO 89/02463.)

Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*e.g.* plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 [Botstein *et al.* (1979) *Gene* 8:17-24], pCU1 [Brake *et al.* (1984) *Proc. Natl. Acad. Sci USA* 81:4642-4646], and YRp17 [Stinchcomb *et al.* (1982) *J. Mol. Biol.* 158:157]. In addition, a replicon may be either a high or low copy number plasmid. A high copy

number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Enter a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See *e.g.* Brake *et al.*, *supra*.

- 5 Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome [Orr-Weaver *et al.* (1983) *Methods in Enzymol.* 101:228-245]. An integrating vector may be  
10 directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al.*, *supra*. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced [Rine *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:6750]. The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the  
15 expression construct in the vector, which can result in the stable integration of only the expression construct.

- Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable  
20 marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions [Butt *et al.* (1987) *Microbiol. Rev.* 51:351].

- Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or  
25 developed into an integrating vector, as described above.

- Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors have been developed for, *inter alia*, the following yeasts: *Candida albicans* [Kurtz, *et al.* (1986) *Mol. Cell. Biol.* 6:142]; *Candida maltosa* [Kunze, *et al.* (1985) *J. Basic Microbiol.* 25:141]; *Hansenula polymorpha* [Gleeson, *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302]; *Kluyveromyces fragilis* [Das, *et al.* (1984) *J. Bacteriol.* 158:1165]; *Kluyveromyces lactis* [De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:737; Van den Berg *et al.* (1990) *Bio/Technology* 8:135]; *Pichia guilliermondii* [Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141]; *Pichia pastoris* [Cregg, *et al.* (1985) *Mol. Cell. Biol.* 5:3376; US Patent Nos. 4,837,148 and 4,929,555]; *Saccharomyces cerevisiae* [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163]; *Schizosaccharomyces pombe* [Beach and Nurse (1981) *Nature* 300:706]; and *Yarrowia lipolytica* [Davidow, *et al.* (1985) *Curr. Genet.* 10:380471 Gaillardin, *et al.* (1985) *Curr. Genet.* 10:49].

- Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See *e.g.* [Kurtz *et al.* (1986) *Mol. Cell. Biol.* 6:142; Kunze  
40 *et al.* (1985) *J. Basic Microbiol.* 25:141; Candida]; [Gleeson *et al.* (1986) *J. Gen. Microbiol.* 132:3459;



- Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302; Hansenula]; [Das *et al.* (1984) *J. Bacteriol.* 158:1165; De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:1165; Van den Berg *et al.* (1990) *BioTechnology* 8:135; Kluyveromyces]; [Cregg *et al.* (1985) *Mol. Cell. Biol.* 5:3376; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; US Patents 4,837,148 & 4,929,555; Pichia]; [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163 Saccharomyces]; [Beach & Nurse (1981) *Nature* 300:706; Schizosaccharomyces]; [Davidow *et al.* (1985) *Curr. Genet.* 10:39; Gaillardin *et al.* (1985) *Curr. Genet.* 10:49; Yarrowia].

#### Pharmaceutical Compositions

Pharmaceutical compositions can comprise polypeptides and/or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgement of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

### Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

- 5 Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (e.g. see WO98/20734), needles, and gene guns or hypodermis. Dosage treatment may be a single dose schedule or a multiple dose schedule.

### Vaccines

- 10 Vaccines according to the invention may either be prophylactic (i.e. to prevent infection) or therapeutic (i.e. to treat disease after infection).

Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, etc. pathogens.

- Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59™ (WO 90/14837; Chapter 10 in *Vaccine design: the subunit and adjuvant approach*, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi: Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphoryl lipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (3) saponin adjuvants, such as Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (e.g. gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc; and (6) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59™ are preferred.

As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), etc.

- 5 The immunogenic compositions (e.g. the immunising antigen/immunogen/polypeptide/protein/ nucleic acid, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

- Typically, the immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The  
10 preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

- Immunogenic compositions used as vaccines comprise an immunologically effective amount of the antigenic or immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in  
15 a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated (e.g. nonhuman primate, primate, etc.), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be  
20 determined through routine trials.

- The immunogenic compositions are conventionally administered parenterally, e.g. by injection, either subcutaneously, intramuscularly, or transdermally/transcutaneously (e.g. WO98/20734). Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be  
25 administered in conjunction with other immunoregulatory agents.

As an alternative to protein-based vaccines, DNA vaccination may be employed [e.g. Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648; see later herein].

#### Gene Delivery Vehicles

- Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention, to  
30 be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence *in vivo* can be either constitutive or regulated.

- The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences.  
35 The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

- Retroviral vectors are well known in the art and we contemplate that any retroviral gene therapy vector is employable in the invention, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB-9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses e.g. MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, 5 Second Edition, Cold Spring Harbor Laboratory, 1985.

- Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.
- 10 These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.
- 15 Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (e.g. HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.
- 20 Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC No. VR-590), Kirsten, Harvey Sarcoma Virus and Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from 25 depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

- Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, 30 WO90/02806, WO90/07936, WO94/03622, WO93/25698, WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) 35 *Human Gene Therapy* 1.

- Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, 40 WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671,

WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (i.e. there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed in Carter US Patent 4,797,368 and Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470. Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 & WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in US Serial No. 08/405,627, filed March 15, 1995, WO94/21792, WO92/10578, WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems.

Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, *Nature* 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86, Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,037 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and *Nature* (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchsacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzyllagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example ATCC VR-925; Trinit virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No.08/240,030, filed May 9, 1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem.* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in US 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585.

Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in US 5,149,655; use of ionizing radiation for activating transferred gene, as described in US 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, *Biochemistry*, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprises therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

#### Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for recombinant protein expression. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (*e.g.* see WO98/20734), needles, and gene guns or hypodermis. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in *e.g.* WO93/14778. Examples of cells useful in *ex vivo* applications include, for example, stem cells, particularly hematopoietic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

5 Polynucleotide and polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

A. Polypeptides

10 One example are polypeptides which include, without limitation: asioloosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of plasmodium falciparum known as RII.

15 B. Hormones, Vitamins, etc.

Other groups that can be included are, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C. Polyalkylenes, Polysaccharides, etc.

20 Also, polyalkylene glycol can be included with the desired polynucleotides/polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide)

D. Lipids, and Liposomes

25 The desired polynucleotide/polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

30 Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy]propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See,



also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See e.g. Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77; Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348; Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

## E. Lipoproteins

In addition, lipoproteins can be included with the polynucleotide/polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, & E, over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, & E apoproteins, LDL comprises apoprotein B; HDL comprises apoproteins A, C, & E.

The amino acid of these apoproteins are known and are described in, for example, Breslow (1985) *Annu Rev. Biochem* 54:699; Law (1986) *Adv. Exp Med. Biol.* 151:162; Chen (1986) *J Biol Chem* 261:12918; Kane (1980) *Proc Natl Acad Sci USA* 77:2465; and Utermann (1984) *Hum Genet* 65:232.

Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in

conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol.* (*supra*); Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J. Clin. Invest* 64:743-750. Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acta* 30: 443. Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Technologies, Inc., Stoughton, Massachusetts, USA. Further description of lipoproteins can be found in Zuckermann *et al.* PCT/US97/14465.

#### 10 F. Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide/polypeptide to be delivered.

Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both *in vitro*, *ex vivo*, and *in vivo* applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples include histones, protamines, human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as (X174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic acid condensing agents. Briefly, transcriptional factors such as C/EBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and putrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

Synthetic polycationic agents which are useful include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides/polypeptides.

#### Nucleic Acid Hybridisation

"Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the stringency of the washing conditions following hybridization. See Sambrook *et al.* [*supra*] vol.2, chap.9, pp.9.47 to 9.57.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated  $T_m$  of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1 µg for a plasmid or phage digest to  $10^{-9}$  to  $10^{-8}$  g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 µg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of  $10^4$  cpm/µg. For a single-copy mammalian gene a conservative approach would start with 10 µg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than  $10^4$  cpm/µg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature ( $T_m$ ) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$T_m = 81 + 16.6(\log_{10} C_i) + 0.4[\%(G + C)] - 0.6(\% \text{ formamide}) - 600/n - 1.5(\% \text{ mismatch}).$$

where  $C_i$  is the salt concentration (monovalent ions) and  $n$  is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138: 267-284).

In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (*ie.* stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and

reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

#### Nucleic Acid Probe Assays

Methods such as PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes according to the invention can determine the presence of cDNA or mRNA. A probe is said to "hybridize" with a sequence of the invention if it can form a duplex or double stranded complex, which is stable enough to be detected.

The nucleic acid probes will hybridize to the Chlamydial nucleotide sequences of the invention (including both sense and antisense strands). Though many different nucleotide sequences will encode the amino acid sequence, the native Chlamydial sequence is preferred because it is the actual sequence present in cells. mRNA represents a coding sequence and so a probe should be complementary to the coding sequence; single-stranded cDNA is complementary to mRNA, and so a cDNA probe should be complementary to the non-coding sequence.

The probe sequence need not be identical to the Chlamydial sequence (or its complement) — some variation in the sequence and length can lead to increased assay sensitivity if the nucleic acid probe can form a duplex with target nucleotides, which can be detected. Also, the nucleic acid probe can include additional nucleotides to stabilize the formed duplex. Additional Chlamydial sequence may also be helpful as a label to detect the formed duplex. For example, a non-complementary nucleotide sequence may be attached to the 5' end of the probe, with the remainder of the probe sequence being complementary to a Chlamydial sequence. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the a Chlamydial sequence in order to hybridize therewith and thereby form a duplex which can be detected.

The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains at least 10-20 nucleotides, preferably 15-25, and more preferably  $\geq 30$  nucleotides, although it may be shorter than this. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

Probes may be produced by synthetic procedures, such as the triester method of Matteucci *et al.* [*J. Am. Chem. Soc.* (1981) 103:3185], or according to Urdea *et al.* [*Proc. Natl. Acad. Sci. USA* (1983) 80: 7461], or using commercially available automated oligonucleotide synthesizers.

The chemical nature of the probe can be selected according to preference. For certain applications, DNA or RNA are appropriate. For other applications, modifications may be incorporated *e.g.* backbone modifications, such as phosphorothioates or methylphosphonates, can be used to increase *in vivo* half-life, alter RNA affinity, increase nuclease resistance *etc.* [*e.g.* see Agrawal & Iyer (1995) *Curr Opin Biotechnol* 6:12-19; Agrawal (1996) *TIBTECH* 14:376-387]; analogues such as peptide nucleic acids may also be used [*e.g.* see Corey (1997) *TIBTECH* 15:224-229; Buchardt *et al.* (1993) *TIBTECH* 11:384-386].

Alternatively, the polymerase chain reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in: Mullis *et al.* [*Meth. Enzymol.* (1987) 155: 335-350]; US patents 4,683,195 & 4,683,202. Two 'primers' hybridize with the target nucleic acids and are used to prime the reaction. The primers can comprise sequence that does not hybridize to the sequence of the amplification target (or its

complement) to aid with duplex stability or, for example, to incorporate a convenient restriction site. Typically, such sequence will flank the desired Chlamydial sequence.

- A thermostable polymerase creates copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a threshold amount of target nucleic acids are generated by the polymerase, they can be detected by more traditional methods, such as Southern blots. When using the Southern blot method, the labelled probe will hybridize to the Chlamydial sequence (or its complement).

- Also, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook *et al* [supra]. mRNA, or cDNA generated from mRNA using a polymerase enzyme, can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labelled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected. Typically, the probe is labelled with a radioactive moiety.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-189 show data pertaining to examples 1-189.

Figure 190 shows a representative 2D gel of proteins in elementary bodies.

- Figure 191 shows an alignment of sequences in five (six) proteins of the invention.

### EXAMPLES

The examples indicate *C.pneumoniae* proteins, together with evidence to support the view that the proteins are useful antigens for vaccine production and development or for diagnostic purposes. This evidence takes the form of:

- Computer prediction based on sequence information from CWL029 strain (*e.g.* using the PSORT algorithm available from [www.psорт.nibb.ac.jp](http://www.psорт.nibb.ac.jp)).
  - Data on recombinant expression and purification of the proteins cloned from IOL207 strain.
  - Western blots to demonstrate immunoreactivity in serum (typically a blot of an EB extract of *C.pneumoniae* strain FB/96 stained with mouse antiserum against the recombinant protein).
  - FACS analysis of *C.pneumoniae* bacteria or purified EBs to confirm accessibility of the antigen to the immune system (see also table III).
  - An indication if the protein was identified by MALDI-TOF from a 2D gel electrophoresis map of proteins from purified elementary bodies from strain FB/96. This confirms that the protein is expressed *in vivo* (see also table V).
- Various tests can be used to assess the *in vivo* immunogenicity of the proteins identified in the examples. For example, the proteins can be expressed recombinantly and used to screen patient sera by immunoblot. A positive reaction between the protein and patient serum indicates that the patient has previously mounted an immune response to the protein in question *ie.* the protein is an immunogen. This method can also be used to identify immunodominant proteins.

The recombinant protein can also be conveniently used to prepare antibodies *e.g.* in a mouse. These can be used for direct confirmation that a protein is located on the cell-surface. Labelled antibody (*e.g.* fluorescent labelling for FACS) can be incubated with intact bacteria and the presence of label on the bacterial surface confirms the location of the protein.

- 5 In particular, the following methods (A) to (O) were used to express, purify and biochemically characterise the proteins of the invention:

#### CLONING OF CPN ORFs FOR EXPRESSION IN *E. COLI*

ORFs of *Chlamydia pneumoniae* (Cpn) were cloned in such a way as to potentially obtain three different kind of proteins:

- 10 a) proteins having an hexa-histidine tag at the C-terminus (epn-His)  
 b) proteins having a GST fusion partner at the N-terminus (Gst-cpn)  
 c) proteins having both hexa-histidine tag at the C-terminus and GST at the N-terminus (GST/His fusion; NH<sub>2</sub>-GST-cpn-(His)<sub>6</sub>-COOH)

- The type a) proteins were obtained upon cloning in the pET21b+ (Novagen). The type b) and c) proteins were obtained upon cloning in modified pGEX-KG vectors [Guan & Dixon (1991) *Anal. Biochem.* 192:262]. For instance pGEX-KG was modified to obtain pGEX-NN, then by modifying pGEX-NN to obtain pGEX-NNH. The Gst-cpn and Gst-cpn-His proteins were obtained in pGEX-NN and pGEX-NNH respectively.

- The modified versions of pGEX-KG vector were made with the aim of allowing the cloning of single amplification products in all three vectors after only one double restriction enzyme digestion and to minimise the presence of extraneous amino acids in the final recombinant proteins.

#### (A) Construction of pGEX-NN and pGEX-NNH expression vectors

- Two couples of complementary oligodeoxyribonucleotides were synthesised using the DNA synthesiser ABI394 (Perkin Elmer) and the reagents from Cruachem (Glasgow, Scotland). Equimolar amounts of the oligo pairs (50 ng each oligo) were annealed in T4 DNA ligase buffer (New England Biolabs) for 10 min in a final volume of 50µl and then were left to cool slowly at room temperature. With the described procedure the following DNA linkers were obtained:

##### gexNN linker:

- 30 NdeI NheI XmaI EcoRI NcoI SalI XhoI SacI NotI  
 GATCCCATATGCTAGCCCGGGGAATTGGTCCATGGAGTGAGTCGACTGACTGAGTGATCGAGCTCCTGAGCGCGCATGAA  
 GGTAATACCGATCGGGCCCTTAAGCAGGTACCTCACTCAGCTGACTGAGCTCCTAGCTCGAGGATCGCGGGGATCTTCGA

##### gexNNH linker:

- 35 HindIII NotI XhoI --Hexa-Histidine--  
 TCGACAAGCTTGGCGCCGCACTCGAGCATCACCATCACCATGAT  
 GTTCGAACGCGCGGCTGAGCACGTAGAGGTAGTGTAGTACTATCGA

The plasmid pGEX-KG was digested with BamHI and HindIII and 100 ng were ligated overnight at 16 °C to the linker gexNN with a molar ratio of 3:1 linker/plasmid using 200 units of T4 DNA ligase

(New England Biolabs). After transformation of the ligation product in *E. coli* DH5, a clone containing the pGEX-NN plasmid, having the correct linker, was selected by means of restriction enzyme analysis and DNA sequencing.

The new plasmid pGEX-NN was digested with SalI and HindIII and ligated to the linker gexNNH. After transformation of the ligation product in *E. coli* DH5, a clone containing the pGEX-NNH plasmid, having the correct linker, was selected by means of restriction enzyme analysis and DNA sequencing.

### (B) Chromosomal DNA preparation

The chromosomal DNA of elementary bodies (EB) of *C. pneumoniae* strain 10L-207 was prepared by adding 1.5 ml of lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 0.6 % SDS, 100 µg/ml Proteinase K, pH 8) to 450 µl EB suspension (400,000/µl) and incubating overnight at 37 °C. After sequential extraction with phenol, phenol-chloroform, and chloroform, the DNA was precipitated with 0.3 M sodium acetate, pH 5.2 and 2 volumes of absolute ethanol. The DNA pellet was washed with 70 % ethanol. After solubilization with distilled water and treatment with 20 µg/ml RNase A for 1 hour at RT, the DNA was extracted again with phenol-chloroform, alcohol precipitated and suspended with 300 µl 1 mM Tris-HCl pH 8.5. The DNA concentration was evaluated by measuring OD<sub>260</sub> of the sample.

### (C) Oligonucleotide design

Synthetic oligonucleotide primers were designed on the basis of the coding sequence of each ORF using the sequence of *C. pneumoniae* strain CWL029. Any predicted signal peptide were omitted, by deducing the 5' end amplification primer sequence immediately downstream from the predicted leader sequence. For most ORFs, the 5' tail of the primers (table I) included only one restriction enzyme recognition site (NdeI, or NheI, or SpeI depending on the gene's own restriction pattern); the 3' primer tails (tableI) included a XhoI or a NotI or a HindIII restriction site.

5' tails		3' tails	
NdeI	5' GTGCGTCATATG 3'	XhoI	5' GCGTCTCGAG 3'
NheI	5' GTGCGTGCTAGC 3'	NotI	5' ACTCGTAGCGGCCGC 3'
SpeI	5' GTGCGTACTAGT 3'	HindIII	5' GCGTAAGCTT 3'

**Table I.** Oligonucleotide tails of the primers used to amplify Cpn genes.

As well as containing the restriction enzyme recognition sequences, the primers included nucleotides which hybridized to the sequence to be amplified. The number of hybridizing nucleotides depended on the melting temperature of the primers which was determined as described [(Breslauer *et al.* (1986) *PNAS USA* 83:3746-50)]. The average melting temperature of the selected oligos was 50-55°C for the hybridizing region alone and 65-75°C for the whole oligos. Table II shows the forward and reverse primers used for each amplification.

**(D) Amplification**

The standard PCR protocol was as follow: 50 ng genomie DNA were used as template in the presence of 0,2  $\mu$ M each primer, 200  $\mu$ M each dNTP, 1,5 mM  $MgCl_2$ , 1x PCR buffer minus Mg (Gibco-BRL), and 2 units of Taq DNA polymerase (Platinum Taq, Gibco-BRL) in a final volume of 100  $\mu$ l. Each sample underwent a double-step amplification: the first 5 cycles were performed using as the hybridizing temperature the one of the oligos excluding the restriction enzyme tail, followed by 25 cycles performed according to the hybridization temperature of the whole lenght primers. The standard cycles were as follow:

denaturation : 94 °C, 2 min

denaturation: 94 °C, 30 seconds	}	5 cycles
hybridization: 51 °C, 50 seconds		
elongation: 72 °C, 1 min or 2 min and 40 sec		

denaturation: 94 °C, 30 seconds	}	25 cycles
hybridization: 70 °C, 50 seconds		
elongation: 72 °C, 1 min or 2 min and 40 sec		

72 °C, 7 min

4 °C

The elongation time was 1 min for ORFs shorter than 2000 bp, and 2 min and 40 seconds for ORFs longer than 2000 bp. The amplifications were performed using a Gene Amp PCR system 9600 (Perkin Elmer).

To check the amplification results, 4  $\mu$ l of each PCR product was loaded onto 1-1.5 agarose gel and the size of amplified fragments compared with DNA molecular weight standards (DNA markers III or IX, Roche). The PCR products were loaded on agarose gel and after electrophoresis the right size bands were excised from the gel. The DNA was purified from the agarose using the Gel Extraction Kit (Qiagen) following the instruction of the manufacturer. The final elution volume of the DNA was 50  $\mu$ l TE (10 mM Tris-HCl, 1 mM EDTA, pH 8). One  $\mu$ l of each purified DNA was loaded onto agarose gel to evaluate the yield.

**(E) Digestion of PCR fragments**

One-two  $\mu$ g of purified PCR product were double digested overnight at 37 °C with the appropriate restriction enzymes (60 units of each enzyme) using the appropriate restriction buffer in 100  $\mu$ l final volume. The restriction enzymes and the digestion buffers were from New England Biolabs. After



purification of the digested DNA (PCR purification Kit, Qiagen) and elution with 30 µl TE, 1 µl was subjected to agarose gel electrophoresis to evaluate the yield in comparison to titrated molecular weight standards (DNA markers III or IX, Roche).

#### (F) Digestion of the cloning vectors (pET21b+, pGEX-NN, and pGEX-NNH)

- 5 10 µg of plasmid was double digested with 100 units of each restriction enzyme in 400 µl reaction volume in the presence of appropriate buffer by overnight incubation at 37 °C. After electrophoresis on a 1% agarose gel, the band corresponding to the digested vector was purified from the gel using the Qiagen Qiaex II Gel Extraction Kit and the DNA was eluted with 50 µl TE. The DNA concentration was evaluated by measuring OD<sub>260</sub> of the sample.

#### 10 (G) Cloning

75ng of the appropriately digested and purified vectors and the digested and purified fragments corresponding to each ORF, were ligated in final volumes of 10-20 µl with a molar ratio of 1:1 fragment/vector, using 400 units T4 DNA ligase (New England Biolabs) in the presence of the buffer supplied by the manufacturer. The reactions were incubated overnight at 16 °C.

- 15 Transformation in *E. coli* DH5 competent cells was performed as follow: the ligation reaction was mixed with 200 µl of competent DH5 cells and incubated on ice for 30 min and then at 42 °C for 90 seconds. After cooling on ice, 0.8 ml LB was added and the cells were incubated for 45 min at 37 °C under shaking. 100 and 900 µl of cell suspensions were plated on separate plates of agar LB 100 µg/ml Ampicillin and the plates were incubated overnight at 37 °C. The screening of the transformants was done by growing randomly chosen clones in 6 ml LB 100 µg/ml Ampicillin, by extracting the DNA using the Qiagen Qiaprep Spin Miniprep Kit following the manufacturer instructions, and by digesting 2 µl of plasmid miniprep with the restriction enzymes specific for the restriction cloning sites. After agarose gel electrophoresis of the digested plasmid mini-preparations, positive clones were chosen on the basis of the correct size of the restriction fragments, as evaluated by comparison with appropriate molecular weight markers (DNA markers III or IX, Roche).

#### (H) Expression

- 1 µl of each right plasmid mini-preparation was transformed in 200 µl of competent *E. coli* strain suitable for expression of the recombinant protein. All pET21b+ recombinant plasmids were transformed in BL21 DE3 (Novagen) *E. coli* cells, whilst all pGEX-NN and all pGEX-NNH recombinant plasmids were transformed in BL21 cells (Novagen). After plating transformation mixtures on LB/Amp agar plates and incubation overnight at 37 °C, single colonies were inoculated in 3 ml LB 100 µg/ml Ampicillin and grown at 37 °C overnight. 70 µl of the overnight culture was inoculated in 2 ml LB/Amp and grown at 37 °C until OD<sub>600</sub> of the pET clones reached the 0,4-0,8 value or until OD<sub>600</sub> of the pGEX clones reached the 0,8-1 value. Protein expression was then

induced by adding IPTG (Isopropyl  $\beta$ -D thio-galacto-pyranoside) to the mini-cultures. pET clones were induced using 1 mM IPTG, whilst pGEX clones were induced using 0.2 mM IPTG. After 3 hours incubation at 37 °C the final OD<sub>600</sub> was checked and the cultures were cooled on ice. After centrifugation of 0.5 ml culture, the cell pellet was suspended in 50  $\mu$ l of protein Loading Sample Buffer (60 mM TRIS-HCl pH 6.8, 5% w/v SDS, 10% v/v glycerol, 0.1% w/v Bromophenol Blue, 100 mM DTT) and incubated at 100 °C for 5 min. A volume of boiled sample corresponding to 0.1 OD<sub>600</sub> culture was analysed by SDS-PAGE and Coomassie Blue staining to verify the presence of induced protein band.

#### PURIFICATION OF THE RECOMBINANT PROTEINS

- Single colonies were inoculated in 25 ml LB 100  $\mu$ g/ml Ampicillin and grown at 37 °C overnight. The overnight culture was inoculated in 500 ml LB/Amp and grown under shaking at 25 °C until OD<sub>600</sub> 0.4-0.8 value for the pET clones, or until OD<sub>600</sub> 0.8-1 value for the pGEX clones. Protein expression was then induced by adding IPTG to the cultures. pET clones were induced using 1 mM IPTG, whilst pGEX clones were induced using 0.2 mM IPTG. After 4 hours incubation at 25 °C the final OD<sub>600</sub> was checked and the cultures were cooled on ice. After centrifugation at 6000 rpm (JA10 rotor, Beckman), the cell pellet was processed for purification or frozen at -20 °C.

#### (I) Procedure for the purification of soluble His-tagged proteins from *E.coli*

1. Transfer the pellets from -20°C to ice bath and reconstitute with 10 ml 50 mM NaHPO<sub>4</sub> buffer, 300 mM NaCl, pH 8.0, pass in 40-50 ml centrifugation tubes and break the cells as per the following outline:
2. Break the pellets in the French Press performing three passages with in-line washing.
3. Centrifuge at about 30-40000 x g per 15-20 min. If possible use rotor JA 25.50 (21000 rpm, 15 min.) or JA-20 (18000 rpm, 15 min.)
4. Equilibrate the Poly-Prep columns with 1 ml Fast Flow Chelating Sepharose resin with 50 mM phosphate buffer, 300 mM NaCl, pH 8.0.
5. Store the centrifugation pellet at -20°C, and load the supernatant in the columns.
6. Collect the flow through.
7. Wash the columns with 10 ml (2 ml + 2 ml + 4 ml) 50 mM phosphate buffer, 300 mM NaCl, pH 8.0.
8. Wash again with 10 ml 20 mM imidazole buffer, 50 mM phosphate, 300 mM NaCl, pH 8.0.
9. Elute the proteins bound to the columns with 4.5 ml (1.5 ml + 1.5 ml + 1.5 ml) 250 mM imidazole buffer, 50 mM phosphate, 300 mM NaCl, pH 8.0 and collect the 3 corresponding fractions of ~1.5 ml each. Add to each tube 15  $\mu$ l DTT 200 mM (final concentration 2 mM)

10. Measure the protein concentration of the first two fractions with the Bradford method, collect a 10 µg aliquot of proteins from each sample and analyse by SDS-PAGE. (N.B.: should the sample be too diluted, load 21 µl + 7 µl loading buffer).
11. Store the collected fractions at +4°C while waiting for the results of the SDS-PAGE analysis.
- 5 12. For immunisation prepare 4-5 aliquots of 100 µg each in 0,5 ml in 40% glycerol. The dilution buffer is the above elution buffer, plus 2 mM DTT. Store the aliquots at -20°C until immunisation.

#### **(J) Purification of His-tagged proteins from Inclusion bodies**

Purifications were carried out essentially according the following protocol:

- 10 1. Bacteria are collected from 500 ml cultures by centrifugation. If required store bacterial pellets at -20°C. For extraction, resuspend each bacterial pellet in 10 ml 50 mM TRIS-HCl buffer, pH 8,5 on an ice bath.
2. Disrupt the resuspended bacteria with a French Press, performing two passages.
3. Centrifuge at 35000 x g for 15 min and collect the pellets. Use a Beckman rotor JA 25.50 (21000 rpm, 15 min.) or JA-20 (18000 rpm, 15 min.).
- 15 4. Dissolve the centrifugation pellets with 50 mM TRIS-HCl, 1 mM TCEP {Tris(2-carboxyethyl)-phosphine hydrochloride, Pierce} , 6M guanidium chloride, pH 8,5. Stir for ~ 10 min. with a magnetic bar.
5. Centrifuge as described above, and collect the supernatant..
- 20 6. Prepare an adequate number of Poly-Prep (Bio-Rad) columns containing 1 ml of Fast Flow Chelating Sepharose (Pharmacia) saturated with Nichel according to manufacturer recommendations.. Wash the columns twice with 5 ml of H<sub>2</sub>O and equilibrate with 50 mM TRIS-HCl, 1 mM TCEP, 6M guanidinium chloride, pH 8,5.
7. Load the supernatants from step 5 onto the columns, and wash with 5 ml of 50 mM TRIS-HCl buffer, 1 mM TCEP, 6M urea, pH 8,5
- 25 8. Wash the columns with 10 ml of 20 mM imidazole, 50 mM TRIS-HCl , 6M urea, 1 mM TCEP, pH 8,5. Collect and set aside the first 5 ml for possible further controls.
9. Elute the proteins bound to the columns with 4,5 ml of a buffer containing 250 mM imidazole, 50 mM TRIS-HCl, 6M urea, 1 mM TCEP, pH 8,5. Add the elution buffer in three 1,5 ml aliquots, and collect the corresponding 3 fractions. Add to each fraction 15 µl DTT (final concentration 2 mM) .
- 30 10. Measure eluted protein concentration with the Bradford method, and analyze aliquots of ca 10 µg of protein by SDS-PAGE.
11. Store proteins at -20°C in 40% (v/v) glycerol, 50 mM TRIS-HCl, 2M urea, 0,5 M arginine, 2 mM DTT, 0,3 mM TCEP, 83,3 mM imidazole, pH 8,5
- 35

**(K) Procedure for the purification of GST-fusion proteins from *E.coli***

1. Transfer the bacterial pellets from  $-20^{\circ}\text{C}$  to an ice bath and resuspend with 7,5 ml PBS, pH 7,4 to which a mixture of protease inhibitors (COMPLETE™ - Boehringer Mannheim, 1 tablet every 25 ml of buffer) has been added. Transfer to 40-50 ml centrifugation tubes and sonicate according to the following procedure:
  - a) Position the probe at about 0,5 cm from the bottom of the tube
  - b) Block the tube with the clamp
  - c) Dip the tube in an ice bath
  - d) Set the sonicator as follows: Timer  $\rightarrow$  Hold, Duty Cycle  $\rightarrow$  55, Out. Control  $\rightarrow$  6.
  - e) perform 5 cycles of 10 impulses at a time lapse of 1 minute (i.e. one cycle = 10 impulses +  $\sim 45''$  hold; b. 10 impulses +  $\sim 45''$  hold; c. 10 impulses +  $\sim 45''$  hold; d. 10 impulses +  $\sim 45''$  hold; e. 10 impulses +  $\sim 45''$  hold)
2. Centrifuge at about 30-40000 x g for 15-20 min. E.g.: use rotor Beckman JA 25.50 at 21000 rpm, for 15 min.
3. Store the centrifugation pellets at  $-20^{\circ}\text{C}$ , and load the supernatants on the chromatography columns, as follows
4. Equilibrate the Poly-Prep (Bio-Rad) columns with 0,5 ml ( $\cong$  1 ml suspension) of Glutathione-Sephrose 4B resin, wash with 2 ml (1 + 1)  $\text{H}_2\text{O}$ , and then with 10 ml (2 + 4 + 4) PBS, pH 7,4.
5. Load the supernatants on the columns and discard the flow through.
6. Wash the columns with 10 ml (2 + 4 + 4) PBS, pH 7,4.
7. Elute the proteins bound to the columns with 4,5 ml of 50 mM TRIS buffer, 10 mM reduced glutathione, pH 8,0, adding 1,5 ml + 1,5 ml + 1,5 ml and collecting the respective 3 fractions of  $\sim 1,5$  ml each.
8. Measure the protein concentration of the first two fractions with the Bradford method, analyse a 10  $\mu\text{g}$  aliquot of proteins from each sample by SDS-PAGE. (N.B.: if the sample is too diluted load 21  $\mu\text{l}$  (+ 7  $\mu\text{l}$  loading buffer)).
9. Store the collected fractions at  $+4^{\circ}\text{C}$  while waiting for the results of the SDS-PAGE analysis.
10. For each protein destined to the immunisation prepare 4-5 aliquots of 100  $\mu\text{g}$  each in 0,5 ml of 40% glycerol. The dilution buffer is 50 mM TRIS.HCl, 2 mM DTT, pH 8,0. Store the aliquots at  $-20^{\circ}\text{C}$  until immunisation..

**SEROLOGY****(L) Protocol of immunization**

1. Groups of four CD1 female mice aged between 6 and 7 weeks were immunized with 20  $\mu\text{g}$  of recombinant protein resuspended in 100  $\mu\text{l}$ .

2. Four mice for each group received 3 doses with a 14 days interval schedule.
3. Immunization was performed through intra-peritoneal injection of the protein with an equal volume of Complete Freund's Adjuvant (CFA) for the first dose and Incomplete Freund's Adjuvant (IFA) for the following two doses.
- 5 4. Sera were collected before each immunization. Mice were sacrificed 14 days after the third immunization and the collected sera were pooled and stored at -20°C.

**(M) Western blot analysis of Cpn elementary body proteins with mouse sera**

- Aliquots of elementary bodies containing approximately 4 µg of proteins, mixed with SDS loading buffer (1x: 60 mM TRIS-HCl pH 6.8, 5% w/v SDS, 10% v/v glycerin, 0.1% Bromophenol Blue, 100 mM DTT) and boiled 5 minutes at 95° C, were loaded on a 12% SDS-PAGE gel. The gel was run using a SDS-PAGE running buffer containing 250 mM TRIS, 2.5 mM Glycine and 0.1 %SDS. The gel was electroblotted onto nitrocellulose membrane at 200 mA for 30 minutes. The membrane was blocked for 30 minutes with PBS, 3% skimmed milk powder and incubated O/N at 4° C with the appropriate dilution (1/100) of the sera. After washing twice with PBS + 0.1% Tween (Sigma) the membrane was incubated for 2 hours with peroxidase-conjugated secondary anti-mouse antibody (Sigma) diluted 1:3000. The nitrocellulose was washed twice for 10 minutes with PBS + 0.1% Tween-20 and once with PBS and thereafter developed by Opti-4CN Substrate Kit (Biorad).

Lanes shown in Western blots are: (P) = pre-immune control serum; (I) = immune serum.

**(N) FACS analysis of *Chlamydia pneumoniae* elementary bodies with mouse sera**

- 20 1.  $2 \times 10^5$  Elementary Bodies (EB)/well were washed with 200 µl of PBS-0.1%BSA in a 96 wells U bottom plate and centrifuged for 10 min. at 1200rpm, at 4°C.
2. The supernatant was discarded and the E.B. resuspended in 10 µl of PBS-0.1%BSA.
3. 10µl mouse sera diluted in PBS-0.1%BSA were added to the E.B. suspension to a final dilution of 1:400, and incubated on ice for 30 min.
- 25 4. EB were washed by adding 180µl PBS-0.1%BSA and centrifuged for 10min. at 1200rpm, 4°C.
5. The supernatant was discarded and the E.B. resuspended in 10 l of PBS-0.1%BSA.
6. 10µl of a goat anti-mouse IgG, F(ab')<sub>2</sub> fragment specific-R-Phycoerythrin-conjugated (Jackson ImmunoResearch Laboratories Inc., cat.N°115-116-072) was added to the EB suspension to a final dilution of 1:100, and incubated on ice for 30 min. in the dark.
- 30 7. EB were washed by adding 180µl PBS-0.1%BSA and centrifuged for 10min. at 1200rpm, 4°C.
8. The supernatant was discarded and the E.B. resuspended in 150 µl of PBS-0.1%BSA.
9. E.B. suspension was passed through a cytometric chamber of a FACS Calibur (Becton Dickinson, Mountain View, CA USA) and 10.000 events were acquired.

10. Data were analysed using Cell Quest Software (Becton Dickinson, Mountain View, CA USA) by drawing a morphological dot plot (using forward and side scatter parameters) on E.B. signals. An histogram plot was then created on FL2 intensity of fluorescence log scale recalling the morphological region of EB.

- 5 NB: the results of FACS depend not only on the extent of accessibility of the native antigens but also on the quality of the antibodies elicited by the recombinant antigens, which may have structures with a variable degree of correct folding as compared with the native protein structures. Therefore, even if a FACS assay appears negative this does not necessarily mean that the protein is not abundant or accessible on the surface. PorB antigen, for instance, gave negative results in FACS but is a surface-exposed neutralising antigen [Kubo & Stephens (2000) *Mol. Microbiol.* 38:772-780].

#### (O) Mass Spectrometry analysis of two-dimensional electrophoretic protein maps

- Gradient purified EBs from strain FB/96 were solubilized at a final concentration of 5.5mg/ml with immobiline rehydration buffer (7M urea, 2M thiourea, 2% (w/v) CHAPS, 2% (w/v) ASB 14 [Chevallet *et al.* (1998) *Electrophor.* 19:1901-9], 2% (v/v) C.A 3-10NL (Amersham Pharmacia Biotech), 2 mM tributyl phosphine, 65 mM DTT). Samples (250µg protein) were adsorbed overnight on Immobiline DryStrips (7 cm, pH 3-10 non linear). Electrophocusing was performed in a IPGphor Isoelectric Focusing Unit (Amersham Pharmacia Biotech). Before PAGE separation, the focused strips were incubated in 4M urea, 2M thiourea, 30% (v/v) glycerol, 2% (w/v) SDS, 5mM tributyl phosphine 2.5%(w/v) acrylamide, 50mM Tris-HCl pH 8.8, as described [Herbert *et al.* (1998) *Electrophor.* 19:845-51]. SDS-PAGE was performed on linear 9-16% acrylamide gradients. Gels were stained with colloidal Coomassie (Novex, San Diego) [Doherty *et al.* (1998) *Electrophor.* 19:355-63]. Stained gels were scanned with a Personal Densitometer SI (Molecular Dynamics) at 8 bits and 50µm per pixel. Map images were annotated with the software Image Master 2D Elite, version 3.10 (Amersham Pharmacia Biotech). Protein spots were excised from the gel, using an Ettan Spot picker (Amersham Pharmacia Biotech), and dried in a vacuum centrifuge. In-gel digestion of samples for mass spectrometry and extraction of peptides were performed as described by Wilm *et al.* [Nature (1996) 379:466-9]. Samples were desalted with a ZIP TIP (Millipore), eluted with a saturated solution of alpha-cyano-4-hydroxycinnamic acid in 50% acetonitrile, 0.1% TFA and directly loaded onto a SCOUT 381 multiprobe plate (Bruker). Spectra were acquired on a Bruker Biflex II MALDI-TOF. Spectra were calibrated using a combination of known standard peptides, located in spots adjacent to the samples. Resulting values for monoisotopic peaks were used for database searches using the computer program Mascot (www.matrixscience.com). All searches were performed using an error of 200-500ppm as constraint. A representative gel is shown in Figure 190.

#### Example 1

- 35 The following *C.pneumoniae* protein (P<sub>TD</sub> 4376552) was expressed <SEQ ID 1; cp6552>:

1 MRKKLSLLVG LTFVLSSCHK EDAQNKIRIV ASPTPHAEILL ESQBEARDEL

51 GIKLKILPVD DYRIPNRLLL DKQVDANYQ HQAFIDDECE RYDCKGELVV  
 101 IAKVHLEPQA IYSKKHSSLE RLKSKQKILTI AIPVDRTNAQ RALHLEECG  
 151 LVCKGFPANL NMTAKDVCGR ENRSINILEV SAPLLVGSPL DVAADVIPGN  
 5 201 FAIAANLSPK KDKSLCLEDELS VSKYTNLVI RSEDVGSPEM IRLKQLKQSP  
 251 SVQHFFDFKY HGNILTTMQD NG\*

A predicted signal peptide is highlighted.

The cp6552 nucleotide sequence <SEQ ID 2> is:

1 ATGAAAAAA AATTATCAT ACTTGTAGGT TTAATTTTGT TTTGAGTTC  
 51 TTGCCATAAG GAAGATGCTC AGAATAAAAT ACGTATTGTA GCCAGTCCGA  
 101 CACCTCATGC GGAATTATTG GAGAGTTTAC AGGAAGAGGC TAAAGATCTT  
 151 GGAATCAAGC TGAATAACTT TCCAGTAGAT GATTATCGTA TTCTAATCG  
 201 TTTGCTTTTG GATAAACAGT TAGATGCAAA TTACTTTCAA CATCAAGCTT  
 251 TTCCTGATGA CGAATGCGAG CGTTATGAT GTAAAGGTGA ATTAGTTGTT  
 301 ATCGCTAAAG TTCATTGGGA ACCTCAAGCA ATTTATTCTA AGAAACATCT  
 351 TTCCTTAGAG CGCTTAAAA GCCAGAAGAA ACTGACTATA CGGATTCCTG  
 401 TGGATCGTAC GAATGCTCAG CGTGTCTAC ACTTGTTAGA AGAGTGGCGA  
 451 CTCATTGTIT TCAAGAGGCC TGCTAAATTA AATATGACAG CTAAGAGTGT  
 501 CTGTGGGAAA GAAATAGAAA GTATCAACAT ATTAGAGGTG TCAGCTCCTC  
 551 TTCCTGTGCG ATCTCTTCCT GACGTTGATG CTGCTGTCA TCTGGGAAT  
 601 TTTGCTATAG CAGCAAAACCT TTCTCCAAAG AAGATAGTCT TTTGTTTAGA  
 651 GGATCTTTGC GTATCTAAGT ATACAAACCT TGTGTGCAAT CGTCTGAAG  
 701 ACGTAGGTTT TCCTAAAAAT ATAAAAATAC AGAAGCTGTT TCAATCTCCT  
 751 TCTGTACAAC ATTTTITTTGA TACAAAATAT CATGGGAATA TTTTGACAA  
 801 GACTCAAGAC AATGGTTAG

25 The PSORT algorithm predicts an inner membrane location (0.127).

The protein was expressed in *E. coli* and purified as a his-tag product, as shown in Figure 1A, and also as a GST-fusion. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 1B) and for FACS analysis (Figure 1C).

The cp6552 protein was also identified in the 2D-PAGE experiment (Cpn0278).

30 These experiments show that cp6552 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

## Example 2

The following *C. pneumoniae* protein (PID 4376736) was expressed <SEQ ID 3; cp6736>:

1 **MKTSIRKFLI** **STTLAPCFAS** **TAFT**VEVIMP SENFDSGSK IPFYTTLSDP  
 51 RGTLCIFSGD LYIANLNDNI SRTSSSCFSN RAGALQLLKG GGVFSLNIR  
 101 SSADGAAISS VITQNPCLCF LSPSGFSQMI FDNCELSLTD TSASNVIPHA  
 151 SAIVATFPMI FTNNDSILFQ YNRSAGFGAA IRGTSTIEN TKKSLLFNGN  
 201 GSIISGGLAT GSAAINLINN SAPVIFSTNA TGIYGAAYL TGGSMITSGN  
 251 LSGVLVFNNS SRSGGAIYAN GNVTFNSNSD LTFQNTASFP QNSLPAPTFF  
 301 PTPPAVTPLL GYGALFCTP PATPPPTGVS LTIISGNSVT FLENIASEQG  
 351 GALTGKILSI DSNKSTIFLG NTAGKGGAIA IPESGSLSL ANQGDILFNK  
 401 NLSITSGTPT RNSIHFGKDA KFATLGATGG YTLFYFDPT SDLSAAGAA  
 451 ATVVNPNKAS ADGAYSQTIV FSGETLTATE AATPANATST LNQKLELGG  
 501 TLALRNAGAT NVHNFTQDER SVVIMDAGTT LATNGANNIT DGAITLNKLIV  
 551 INLSDLDGTL AAVVNVQSTN GALTISGTLG LVKNSQDCCD NHGMPNKDLQ  
 601 QVPLSLKAT NTVTTTTFDS LGTNGYQQSP YGYQTWEFT IDTTHTTVTG  
 651 NWKTKGYLPH PERLAPLIPN SLWANVIDLR AVSQASAADG EDVPGQLSI  
 701 TGITNFFHAN HTGDARSYRH MGGGYLINTY TRITPDAALS LGFGQLFTKS  
 751 KXVLVGHGHS NVYFATVYSN ITKSLFGSSR FFSGGTSRVT YSRNENKVT  
 801 SYTKLFKGRS SWSNKNLWGE LEGNLPITLS SRLNLKQII FPKVAEAVYA  
 851 THGGIQENTP EGRIFGHGHL LNVAFVGVGR FPKNSHNRPD FTTIIVAYAP  
 901 DRYRHNPDCT TTLFINGATW TSGINNLTRS TLLVQASSHT SVNDVLEIFG  
 951 HCGCDIRRTS RQYTLDIGSK LRF\*

A predicted signal peptide is highlighted.

The cp6736 nucleotide sequence <SEQ ID 4> is:

```

1  ATGAAACGCT  CTATTCTGTAA  GTTCTTAATT  TCTACCACAC  TGGCGCCATG
51  TTTTGCTTCA  ACAGCGTTTA  CTGTAGAAAT  TATCATGCCT  TCCGGAACCT
101  TTGATGGATC  GAGTGGGAAG  ATTTTTCCTT  ACACAACACT  TTCTGATCCT
151  AGAGGGACAC  TCTGTATTTT  TCTAGSGGAT  CTCTACATGT  CGAATCTTGA
201  TAATGCCATA  TCCAGAACCT  CTTCAGATTG  CTTTAGCAAT  AGGCGGGGAG
251  CACTACAAAT  CTTAGGAAAA  GGTGGSGTTC  TCTCCTCTCT  AAATATCCGT
301  TCTTTCAGCT  ACGGAGCCGC  GATTAGTAGT  GTAATCACC  AAAATCCTGA
351  ACTATGTCCC  TTGAGTTTTT  CAGGATTTAG  TCGATGATC  TTGATTAATC
401  GTGAATCTTT  GACTTCAGAT  ACCTCAGCGA  GTAATGTCT  ACCTCACGCA
451  TCGGCGATTT  ACGCTACAA  GCCCATGTCT  TTTACAAACA  ATGACTCCAT
501  ACTATTCCAA  TACAACCGTT  CTGCGAGATT  TGGAGCTGCC  ATTGAGGGCA
551  CAAGCATCAC  AATAGAAAA  ACGAAAAAGA  GCCTTCTCTT  TAATGTGAAT
601  GGATCCATCT  CTAATGGAGG  GGCCCTCAG  GGAATCTGAG  CGATCAACCT
651  CATCAACAAT  AGCGCTCCTG  TGAATTTTCT  AACGAATGCT  ACAGGATCTT
701  ATGTGGGGGC  TATTTACCTT  ACCGGAGGAT  CTATGCTCAC  CTCTGGGAAC
751  CTCTCAGGAG  TCTTGTTCGT  TAATAATAGC  TCGCGCTCAG  GAGGCGCTAT
801  CTATGCTAAC  GGAATGTCA  CATTTTCTAA  TAACACGAC  CTGACTTTCC
851  AAAACAATAC  AGCATCTCCA  CAAAACCTCT  TACCTGCACC  TACACTCCCA
901  CCTACACCAC  CAGCAGTCAC  TCTTTTGTGA  GGATATGGAG  GCGCATCTCT
951  CTGTACTCCT  CAGCTACCC  CCCACCAAC  AGGTGTAGC  CTGACTATAT
1001  CTGAGAGAAA  CAGCGTTACA  TTCTTAGAAA  ACATTGCTC  CGAACAAGGA
1051  GGAGCCCTCT  ATGGCAAAA  GATCTCTATA  GATCTAAATA  AATCTCAAT
1101  ATTTCTTGGA  AATCAGCTG  GAAAGGAGG  CGTATTGTCT  ATTCCGGAAT
1151  CTGGGGAGCT  CTCTCTATCC  GCAAACTCAG  GTGATATCT  CTTTACAAG
1201  AACTCTAGCA  TCACATAGTG  GACACTTACT  CGCAATAGTA  TTCACTTCGG
1251  AAAAGATGCC  AAGTTTGCCA  CTCTAGGAGC  TACGCAAGGC  TATACCTTAT
1301  ACTTCTATGA  TCCGATTACA  TCTGTGATT  TATCTGCTGC  ATCCGCAACC
1351  GCTACTGTGG  TCGTCAATCC  CAAAGCCAGT  GCAGATGGTG  CGTATTCAAG
1401  GACTATTGTC  TTTTTCAGG  AAACCCCTAC  TGCTAACQAA  GCAGCAACCC
1451  CTGCAATGTC  TACATCTACA  TTAACCAAAA  AGCTAGAAT  TGAAGGCGGT
1501  ACTCTCGCTT  TAAGAAACGG  TGCTACCTTA  ATGTTCATA  ACTTCAAGCA
1551  AGATGAAAAG  TCCGTCGTCA  TCAATGATGC  AGGACCCACA  TTAGCAACTA
1601  CAAATGGAGC  TAATAATACT  GACGCTGCTA  TCACCTTAAA  CAACTTGTA
1651  ATCAATCTGG  ATTCTTTGGA  TGGCACTAAA  GCGGTGTCG  TTAATGTGCA
1701  GAGTACCAAT  GGAGCTCTCA  CTATATCCGG  AACITTAGGA  CTGTGAAAA
1751  ACTCTCAAGA  TTGCTGTGAC  AACCAAGGGA  TGTTTAATAA  AGATTTCAGC
1801  CAAGTTCGGA  TTTTAGAACT  CAAAGCGACT  TCAATACCTG  TTAACCACTAC
1851  GGACTTCAGT  CTCGGCACAA  ACGGCTATCA  GCAATCTCCC  TATGGGTATC
1901  AAGGAACCTG  GGAGTTTACC  ATAGACACGA  CAACCCATAC  GGTACAGGGA
1951  AATTGGAATA  AAACCGGTTA  TCTTCTCAT  CCGGAGCGTC  TTGCTCCCTC
2001  CATTCCTAAT  AGCCTATGGG  CAAACGTCAT  AGATTTACGA  GCTGTAACTG
2051  AAGCGTCAGC  AGCTGATGGC  GAAGATGTCC  CTGGGAAGCA  ACTGAGCATC
2101  ACAGGAATTA  CAAATTTCTT  CCATGCGAAT  CATACCGGTG  ATGCAACGCA
2151  CTACCGCCAT  ATGGGTGGAG  GCTACCTCAT  CAAATCTTAC  ACACGCATCA
2201  CTCCAGATGC  TGCCTTAAGT  CTAGGTTTTG  GACAGCTGTT  TACAAAATCT
2251  AAGGATTAAC  TCGTAGTCA  CGGTCTTCT  AACGTTTAT  TCGCTACAGT
2301  ATACTCTAAC  ATCAACAAGT  CTCTGTTTGG  ATCATCGAGA  TTCTTCTCAG
2351  GAGGCACCTT  TCGAGTTACC  TATAGCCGTA  GCAATGAGCA  AGTAAGAATC
2401  TCAATATACAA  AATTGCTTAA  AGGCGCTGCT  TCTTGGAGTA  ACAATTGCTG
2451  GTTAGGAGAA  CTGGAAGGGA  ACCCTCCCAT  TCTTGGAGTA  TCTGCTATCT
2501  TAAACCTCAA  GCAGATCAT  CCTTTGTAA  AAGCTGAAGT  TGCTTACGGC
2551  ACTCATGGGG  GCATCCAAAG  AAATACCCCC  GAGGCGGAGA  TTTTGGACA
2601  CGGTCACTTA  CTCACGTTG  CAGTTCCGCT  AGGCGTCCGC  TTTGGTAAAA
2651  ATTCTCATAA  TCGACAGAT  TTTTACACTA  TAATCTGATC  CTATGCTCCT
2701  GATGCTTATC  GTCAACAATC  TGATTGCGAT  ACAGCATTAC  CTATTAATGT
2751  AGTACCTGAG  ACCCTCTATG  GGAATRAATCT  AACCAGAAGT  ACTTTGCTAG
2801  TACAAGCATC  CAGCCATATC  TCAGTAAATG  ATGTTCTAGA  GATCTTCGGG
2851  CACTGTGGAT  GTGATATTGG  CAGAACCCTC  COTCAATATA  CTCTAGATAT
2901  AGGAAGCAAA  TTACAGTTTT  AA

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The PSORT algorithm predicts an outer membrane location (0.917).



The protein was expressed in *E.coli* and purified as a his-tag product, as shown in Figure 2A, and also as a GST-fusion. Both proteins were used to immunise mice, whose sera were used in a Western blot (Figure 2B) and for FACS analysis (Figure 2C).

The cp6736 protein was also identified in the 2D-PAGE experiment (Cpn0453) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6736 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 3

The following *C.pneumoniae* protein (PID 4376751) was expressed <SEQ ID 5; cp6751>:

```

10      1  MRPFCEGMLL PFTFVLANEQ LQLPLETYIT LQPEYQAAQF VGPTHNQND
      51 LAIVGNHDF ILDYKYRSN GGALTCNML ISENIGNVFF EKNVCPNMSG
      101 AIYAAQNCIT SKHQYAPTT NLVSDNPTAT AGSLGGALF AINCSTNNL
      151 GQCTFFDMLA LNKGGALYTE TNLISKDNKG PIIIKQWAL NQSDLOGGIV
      201 SGNGLNIEGN SGAIQITENS SGSGGGIFST QTLTISNNK LIBISNSAF
      251 ANNVYGNFNP GGGGLTTTFC TILNRRBVL FNNQSQSNG GAHAKSIII
      301 KENGFPYFLN NATRGALL NLASGSGNGS FILSADNGIT IPNNNTASKH
      351 ALNPPYRNAI HSTPMNMLQI GARPGYRVLF YDPIEHLPS SFPILPNFTF
      401 GHTGTVLFSG EHVHQNPTDE MNFTSYLRNT SELRQGVLA EDGAGLACYK
      451 PFGRGGLLLL GGGAVITTAG TITPTSPSTPI TVGSTITLNI IADPLSILS
      501 PQAQAPKIKI YPTKGTSTYT EDGNPTITIS GTLTLRNSNN EDPYDGLDLG
      551 HSLEKVPLLY IVDVAQKIN SQGLDLSTLN SGHYGYQGI WSTYVWEITP
      601 ITNPTSLIGA NTKHKLIVAN WSLPGYRPH ERREGEITNA LWQAYTALA
      651 GLHLSLSSWE EKHGAASLQG IGLLVHQKDK NGFKGPRSHM TQYSATREAT
      701 SSQSPNFSLG PAQFFSKARE HESQNSTSHI HYFGMCINR TLFKEMIRLS
      751 VSLAYMTSE HTHMTYQGL EGNSSQSFHN HTLAGALSCV FLPPQPHBSL
      801 QIYFFITALA TRGNLAAPQE SGDHAREFSL HRPLTDVSLP VGIKASWKNH
      851 HRVPLVWLTE ISYRSTLYRQ DPELHSLKLI SQGTWITQAT FVTYNALGIK
      901 VKNIMGVFFK VTLSDLSAD ISSSLGHYLV NVASRMRF*

```

A predicted signal peptide is highlighted.

30 The cp6751 nucleotide sequence <SEQ ID 6> is:

```

30      1  ATGCGCTTTT TTTGCTTCGG AATGTTGCTT CTTTCTACIT TTGTATTGGC
      51 TAATGAAGGT CTCCAACTTC CTTTGAGAGC CTATATATACA TTAAGTCCGT
      101 AATATCAAGC AGCCCCCTCA GTAGGGTTTA CTCATAACCA AAATCAAGAT
      151 CTCGCAATTG TCGGGAATCA CAATGATTTT ATCTTTGACT ATAACTACTA
      201 TCGGTGCAAT GGAGGTGCTT TTACCTGTAA GAATCTTCTG ATCTCTGAAA
      251 ATATAGGGAA TGTTCTCTTT GAGAGAATG TCTGTCCCAA TTCTGGCGGG
      301 GCAATTATATG CTGCTCAAAA TTGCACGATC TCCAGAATC AGAACTATGC
      351 ATTTACTACA AACTTGGTCT CTGACATCC TACAGCCACT CGGGGATCAC
      401 TAITGGGTGG AGCTCTCTTT GCCATAAAT GCTCTATATC TAATAACCTA
      451 GGACAGGGAA CTTTCTGTGA CAATCTCGCT TTAATAAAGG GGGGTGCCCT
      501 CTATACTGAG ACGCACTTAT CTATTAAAGA CAATAAAGGC CGGATCATAA
      551 TCAAGCAGAA TCGGGCACTA AATTTCGAGA GTTTAGGAGG AGGGATTTAT
      601 AGTGGGAACCT CTCTAATAT AGAGGGAAAT TCTGGAGCTA TACAGATCAC
      651 AAGCAACTCT TCAGGATCTG GGGGAGGATC ATTTTCTACC CAACACTCA
      701 CGATCTCCCT GAATAAAAAA CTCATAGAAA TCAGTAAAAA TTCCGCGTTC
      751 GCATAAATCT ATGGATCGAA CTTCATCCCA GGAGGAGGAG GTCTTACTAC
      801 CACCTTTTGC ACGATATTGA ACAACCGAGA AGGGGTACTC TTATAACAATA
      851 ACCAAAGCCA GAGCAACGGT GGAGCCATTC ATGCGAAATC TATCATTTATC
      901 AAAGAAAATG GTCCGTGTATA CTTTTTAAAT AACACTGCAC TCGGGGAGG
      951 GGCTCTCCCT AACTTATCAG CAGGTCTCGG AACACGGAAG TCATCTTTAT
      1001 CTCGAGATAA TGGAGATATT ATCTTTAACA ATATACGGCG CTCACAGCAT
      1051 GCCCTCAATC CTCATACAG AACCGCCATT CACTCGACTC CTAATATGAA
      1101 TCTCGAATAA GGAGCCCGCT CCGGCTATCG AGTGTGTGTC TATGATCCCA
      1151 TAGAACATGA GCTCCCTTCC TCCTTCCCCA TACTCTTTAA TTTCGAACAC
      1201 GGTCAATACG GTACAGTTTT ATTTTCAGGG GAACATGTAT ACCGAACATC

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	1251	TACCGATGAA	ATGAATTCT	TTTCCTATTT	AAGGAACACT	TCGGAACACT
	1301	GTCAAGGAGT	CCTTGCTGTT	GAAGAIGSTG	CGGGGCTGGC	CTGCTATAAG
	1351	TTCTTCCAAAC	GAGGAGGGCA	TCTACTTCTA	GGTCAAGGTG	CGGTGATCAC
5	1401	GACAGCAGGA	ACGATTCCEA	CACCATCTTC	AACACCAACG	ACAGTAGGAA
	1451	GTACTATAAC	TTTAAATCAC	ATTGCCATTC	ACCTTCCCTTC	TATTTCTTCT
	1501	TTTCAAGCTC	AGGCTCCAAA	AATTTGGATT	TACCCACAAA	AAACAGGATC
	1551	TACCTATACT	GAGATTCCEA	ACCGACAAT	CACAACTCTCA	GGAACTCTCA
	1601	CCTTACGCAA	CAGCAACAAC	GAGATCCCT	ACGATAGTCT	GGATCTCTGG
	1651	CACCTCTCTG	AGAAAGTTC	CCTCTTPTAT	ATTGTCGATG	TCGCTGCACA
10	1701	AAAAATTAA	TCTTGGCAAC	TGGATCTATC	CACATTAAT	TCTGGCGAAC
	1751	ACTATGGGTA	TCAAGGCATC	TGGTGCAGCT	ATTGGGTAGA	AACTACAACA
	1801	ATCACGAACC	CTACATCTCT	ACTAGGCGCG	AATACAAAAC	ACAAGCTGCT
	1851	CTATGCGAAC	TGGTCTCTCT	TAGGCTACCG	TCCTCATCCC	GAACTGCGAG
	1901	GAGAAFTCAT	TACGAATGCC	TTGTGGCAAT	CGGCATATAC	GGCTCTTGCA
15	1951	GGACTCCAAT	CCCTCTCTCT	CTGGGATGAA	GAGAAGGGTC	ATGCAGCTTC
	2001	CCTACAAAGC	ATTGGTCTTC	TGGTTCATCA	AAAAGACAAA	AACGGTITTA
	2051	AGGGATTTCC	TAGTCATATG	ACAGGTATTA	GTGCTACACC	CGAAGCAACC
	2101	TCTTCTCAA	GTCCGAATTT	CTCTTTAGGA	TTTGCTCAGT	TCTTCCCAAA
20	2151	AGCTAAAGAA	CATGAATCTC	AAAAATAGCA	GTCCCTCTCAC	CACATTTTCT
	2201	CTGGAATGTG	CATAGAAAAT	ACTCTCTTCA	AAGAGTGGAT	ACGCTATCTT
	2251	GTGTCCTCTG	CTATATAGTT	TACCTCGGAA	CATACCCATCA	CAATGTATCA
	2301	GGGTCTCTCG	GAAAGGAACT	CTCAGGGATC	TTTCCACAAC	CATACCTTAG
	2351	CAGGGGCTCT	CTCCTGTGTT	TTCTTACCTC	AACCTCA CGG	CGAGTCCCTG
	2401	CAGATCTATC	CTTTTATATC	TGCCTTAGCC	ATCCGAGGAA	ATCTTGTGTC
25	2451	GTTCAGAGAA	CTTGAGAGAC	ATGCTCGGGA	ATTTCCTCTA	CACCGCCCC
	2501	TAACGGAAGT	CTCCCTCCCT	GTAGGAATCC	CGCTCTCTTG	GAAAGAACAC
	2551	CACCGAGTTC	CCCTAGTCTG	GCTCACAGAA	ATTTCCTATC	GCTCTACTCT
	2601	CTATAGGCAA	GATCCCTGAA	TCCACTCGAA	ATTACTGATT	AGCCAAGGTA
	2651	CGTGGACGAC	CGAGGCCACT	CCTGTGACCT	ACAACTGCTT	AGGGATCAAA
30	2701	GTGAAAATAA	CCATGCAGGT	GTTCCTCTAA	GTCACTCTCT	CCTTAGATTA
	2751	CTCTGCGGAT	ATTTCTCTCT	CCACGCTGAG	TCACTACTTA	AACGTGGCGA
	2801	GTAGAATGAG	ATTTTAA			

The PSORT algorithm predicts an outer membrane location (0.923).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 3A, and also in his-tagged form. The GST-fusion recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 3B) and for FACS analysis (Figure 3C).

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6751 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 4

The following *C. pneumoniae* protein (PID 4376752) was expressed <SEQ ID 7; cp6752>:

	1	MFGMTPAVYS	LQTDLSLEKFA	LERDEEFRTS	PFLDLSLSLT	TGFSPIITFV
	51	GNRNESSQDI	VLSNYKSIDL	ILLMTWAGS	AVSNCNFFLS	NVEDHAFFSK
45	101	NLAIGTGGA	ACQGACTTTR	NRGFLIFFSN	RGLNNASTGG	ETRGGAICAN
	151	GDFTISQNG	TFYFVNNSVN	NWGGALSTNG	KCRIGSNAPD	LLFFNMTAPF
	201	GGGALRSNT	TISDNFRPIY	FKNNCNRNG	AIOTSVTVAI	KNNSSGVIFN
	251	NNTALSGSIN	SGNSGGAIY	ITNLSDIDST	CLLFASGQNI	AFQNEVPIT
	301	FTLTKNSQHV	YFTNNQGNWG	GALMLIQDST	PIEHQHPPTN	PLIFNMNHN
50	351	TFGRYNAIHC	TPNSNLQLCA	NKGVTAFPTD	LRHWLSDIED	RAGWYFYKPT
	401	QQTLLFSAY	IFEASDYERIN	FSSSKNFS	VIINMLAINL	PSLAKGKAP
	451	QGGGLKLGSH	ASASTATANG	ETPSTVSQSG	TLISGPT/LA	NENENDPYDS
	501	TLWIRFLQSS	APFEDNNPT	TLISGPT/LA	YQIINSPYVW	ETPTTNNAS
	551	HLLSLSDVTA	RHINTDNFHP	ESLNATETHYG	ATTPFWQSEH	TFPSLRSYN
55	601	LETANTLYRA	LIANWTPLGY	KVNPEYQDL	APGFRIQSTG	YSLQASSETF
	651	RTGSDIERP	FLEIQGLADG	LFVHQNSIQ		

701	LHQKISLGFA	QFFTRTKEIG	SSNNVSAHNT	VSSLYVELEW	FQEFATSTV
751	LAYGVGDHHL	HSLHPSHQEQ	AECTCYSHWL	AAAGCSGFW	QQKSYLHLSF
801	FVQAIARISH	QTAFFEIGDN	PRKFSVKPFF	YNLTPLPGIO	GRWQSKFHPV
851	TEWILHLSYQ	PVLYQQNPQI	GVTLASGGG	WDILGHNVVR	NALSYKVHNO
901	TALFRSLDLF	LDYQGSVSSS	TSHTHLQAGS	TLKF*	

The cp6752 nucleotide sequence <SEQ ID 8> is:

1	ATGTTCCGGA	TGACTCTCTG	AGTGATAGT	TTACAAACGG	ACTCCCTTGA
51	AAAGTTTCC	TTAGAGAGGG	ATGAAGAGT	TGTCAGGAGC	TTTCTCTCT
101	TAGACTCTCT	CTCCACTCTT	ACAGGATTCT	CTCGATAAAC	TACCTTTGTT
151	GGAAATAGAC	ATAATTCTCT	TCAGACATAT	GTACTTTCTA	ACTACAATCT
201	TATGTATAAC	ATCTCTCTTC	TTTTGACATC	GGCTGGGGGA	GCTGCTCTCT
251	GTATATAATT	CTTATTATAT	AATGTTGAAG	ACCATGCCCT	CTTCAGTAAA
301	AATCTCGCA	TTGGAGCTGG	AGGCGCATTT	GCTGCCAGG	GAGCCTGCAC
351	AATCACGAAG	AATAGAGGAC	CCCTTAATTT	TTTCAGCAAT	CGAGGCTCTA
401	ACAATGCCAG	TACAGGAGGA	GAAACTCTGT	GGGTGCGAT	TGCTGTAAAT
451	GGAGACTTCA	CGATTCTTCA	AAATCAAGGG	ACTTTCTACT	TTGTCAACNA
501	TTCCGTCAC	AACTGGGGAG	GAGCCCTCTC	CACCAATGGA	CAGTCGCCCA
551	TCCAAGACAA	CMGGGCACCT	CTACTCTTTT	TTACAATAC	AGCCCTTAGT
601	GGAGGGGGTG	CGCTTCGTAG	TGAAATATCA	ACGATCTCTG	ATAACACCGG
651	TCCTATTATT	TTTAAAGAACA	ACTGTGGGAA	CAATGGCGGG	GCCATTCAAA
701	CAAGCGTTAT	TGTTGCGATA	AAAATAAAT	CCGGTCTGGT	GATTTTCAAT
751	AAACAACACAG	CGTTATCTGG	TTCGATAAAT	TCAGGAATAG	GTTCAGGAGG
801	GGCGATTAT	ACAAACAAC	TATTCATAGA	CGATAACCTT	GGAACTATT
851	TTTTTCAMTA	TAATTAATCT	ATTCGCGATG	GCGGAGCTAT	CTGTACACNA
901	TTTTTGACAA	TCAAAAATAG	TGGCCACGTA	TATTTTCAAC	ACAATCAAGG
951	AAACTGGGGA	GGTGCTCTTA	TGCTCTTACA	GGACGACACC	TGCTTACTCT
1001	TGCGCGAACA	AGGAAATATC	GCATTTCAAA	ATAATGAGGT	TTTCTCTACC
1051	ACATTTGGTA	GATACAACGC	CATACATTTG	ACACCAATAA	GCAACTTACA
1101	ACTTGGAGCT	AATAAGGGGT	ATACGACTGC	TTTTTTTGAT	CCTATAGAAC
1151	ACCAACATCC	AACTACAAAT	CTCTTAATCT	TTAATCCCAA	TGCGAACCAT
1201	CAGGGAACGA	TCTTATTTTC	TTTCAGCTAT	ATCCCGAAG	CTTCTGACTA
1251	CGAAATAAAT	TCTATTAGCA	GTTCGAAAAA	TACCTCTGAA	CTTCGCAATG
1301	GTGTCCCTCT	TATCCGAGGAT	CGTCCGGGAT	GGCAATTCTA	TAASTTCACT
1351	CAAAAAGGAG	GTATCCTTAA	ATTAGGGCAT	GCGCGAGTGA	TTGCAACAAC
1401	TGCCAATCT	GAGACTCCAT	CAACTAGTGT	AGGCTCCGAT	GTTCATTAAT
1451	TAAACCTTGC	GATTAACTCT	CCCTCGATCT	TAGCAAAAGG	AAAAGCTCCT
1501	ACCTTGTGGA	TCCGTCTCTT	ACAATCTAGT	GCTCCTTTCA	CAGAGGACAA
1551	TAACTCTACA	ATTACTTTAT	CAGGTCTCTT	GACACTTTTA	AATGAGGAAA
1601	ACCGCGATCC	CTACGACAGT	ATAGMTCTCT	CTGAGCCTTT	ACAAAACATT
1651	CATCTTCTTT	CTTTATCGGA	TGTAACAGCA	CGTCATATCA	ATACCGATAA
1701	CTTTTACTCT	GAAAGCTTAA	ATCCGACTGA	GCATTACGGT	TATCAAGGCA
1751	TCTGGTCTTC	TTATTGGGTA	GAGACGATAA	CAACAACAAA	TAACTCTTCT
1801	ATAGAGACGG	CAAAACCTCT	CTACAGAGCT	CTGTATGCCA	ATTGGACTCC
1851	CTTAGGATAT	AAGGTCAACT	CTGAATACCA	AGGAGATGCT	GCTACGACTC
1901	CCCTATTGCA	ATCCTTTTAT	ACTATGTCTT	CTCTATTAA	AAAGTTATAAT
1951	CGAATCTGGT	ATTCTGATAT	CGAGGAGCTT	TTCTTAGAAA	TTCAAGGGAT
2001	TGCCGACGGC	CTCTTTGTCT	ATCAAAATAG	CATCCCGGGG	GCTCCAGGAT
2051	TCCGTATCCA	ATCTACAGGG	TATTCCTTAC	AAAGCATCTC	CGAACTTCTT
2101	TTACATCAGA	AAATCTCCTT	AGGTTTTGCA	CAGTTCTTCA	CCCGACTAAA
2151	AGAAATCGGA	TCAAGCAACA	ACGTCCTGGG	TCACAATACA	GTCTCTTCAC
2201	TTTATGTTGA	GCTTCTGGTG	TTCCAAGAGG	CCCTTCGAAC	ATCCACAGTG
2251	TTAGCGTATG	GCTATGGGGA	CAATCACTCT	CACAGCCTAC	ATCCCTCACA
2301	TCAAGAACAG	CGAGAGGGGA	CGTGTATAG	CCATACATTA	CGACAGCTTA
2351	TGCGCTGPTC	TTTCCCTTGG	CAACGAAAT	CCATCTCTCA	CCTCAGCCCG
2401	TTCTGTCAGG	CAATTGCAAT	ACGTTCTCAC	CAACACAGGT	TCGAAAGAGT
2451	TGGTGACAA	CCCGGAAAGT	TGTGCTCTCA	AAAGCCTTTC	TATTAATCTGA
2501	CTTTACTCT	AGGAATCCAA	GGAATAATGC	AGTCAAAAT	CCAGCTTACT
2551	ACAGAAATGGA	CTCTAGAACT	TCTCTACCAA	CCGTTACTCT	ATCAACAAAA
2601	TCCCCAAATC	GGTGTCAAGC	TAGCTTCGAG	CGGAGGTTCC	TGGGATATCC
2651	TAGGCCATAA	CTATGTTGCG	AATGCTTTAG	GGTACAAAGT	CCCAATCAAA
2701	ACTGCGCTCT	TCCGTTCTCT	CGATCTATTC	TTGAGTTACC	AAGGATCGGT
2751	CTCTCTCTCG	ACATCTAGCG	ACCATCTCCA	AGCAGAGAT	ACCTTAAAT
2801	TCTAA				

The PSORT algorithm predicts a cytoplasmic location (0.138).

The protein was expressed in *E.coli* and purified as a his-tag product, as shown in Figure 4A, and also as a GST-fusion. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (4B) and the his-tagged protein was used for FACS analysis (4C).

The cp6752 protein was also identified in the 2D-PAGE experiment (Cpn0467).

- 5 These experiments show that cp6752 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 5

The following *C.pneumoniae* protein (PID 4376850) was expressed <SEQ ID 9; cp6850>:

10 1 MKKAVLTAAH FCGVSLSSC CRIVDCCFED PCAPSSNCPC EVIRKKERSC  
51 GGNACGSYVF SCSNFCGSTB CNSQSPQVKG CTSFDGRCKQ \*

A predicted signal peptide is highlighted.

The cp6850 nucleotide sequence <SEQ ID 10> is:

1 ATGAAGAAG CTGTTTAAAT TGCTGCAATG TTTTGTGGAG TAGTTAGCTT  
51 AAGTAGCTGC TGCAGCATTC TAGATTGTTG TTTTGAGGAT CCTTGCGCAC  
101 COTCTTCTTG CAATCCTTCT GAAGTAATAA GAAAAAAGA AAGATCTTGC  
151 GCGCGAATG CTGTGTGGTC CTAGTTCCT TCTTGTCTTA ATCCAGTGG  
201 TTCAACAGAG TGTAACTCTC AAGCCACAC AGTTAAAGGT TOTACATCAC  
251 CTGATGGCAG ATGCAACACG TAA

The PSORT algorithm predicts an inner membrane location (0.329).

- 20 The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 5A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 5B) and for FACS analysis (Figure 5B). A his-tagged protein was also expressed.

These experiments show that ep6850 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### 25 Example 6

The following *C.pneumoniae* protein (PID 4376900) was expressed <SEQ ID 11; cp6900>:

1 MKIKFSWKVN FLICLLAVGL IFPGCSRVER EVLVGRDATW FPKQFGIYTS  
51 DTNAPLNDLV SEINYKENLN INIVNQDWVH LPENLDDKKT QGAFTSVLPT  
101 LEMLEHYQFS DPILLTGPVL VVAQDSPYQS IEDLKGRLIG VYKFDSSVLV  
151 AQNIPDAVIS LYQHPVIALE ALTSNCDAL LAPVIEVTAL IETAYKRLK  
201 IISKPLNADG LRLAILKGTN GDLEGFNAG LVKTRSRGKY DAIKQRYRLP

The cp6900 nucleotide sequence <SEQ ID 12> is:

1 GTGAAGATAA AATTCTTCTG GAAGGTAAAT TTTTAAATAT GTTTACTGGC  
51 TGTGGGAGTG ATCTTTTCTG GGTCCTCTCG AGTAAAAGA GAAGTCTCTG  
101 TAGGTCGTGA TGCCACCTGG TTTCCAAAAC AATTCGCCAT TTATACATCC  
151 GATACCAACG CATTTTTAAA GATCTTGTIT TCTGAGATTA ACTATAAGA  
201 GAATCTAAT ATTAATATTG TAAATCAAGA TTGGGTGCAT CTCCTTGAGA  
251 ATTTAGATGA TAAAAGACC CAAGGAGCAT TTACATCTGT ATTGCTTACT  
301 CTTGAGATCG TCGAACACTA TCAATTTTCT GATCCCATTT TACTCACAGG  
351 TCTCTGTCTT GTCTGTGCTC AAGACCTCC TTACCAATCT ATAGAGGATC  
401 TTAAGGTCG TCTTATTGGG GTGTATTAAG TTGACTCTTC AGTCTTGTA  
451 GCTCAAAATA TCCCTAGACC TGGTATTAGC CTCTACCAAC ATGTCTCAAT  
501 AGCATTTGGA GCTTAAACAT CGAATTGTTA CGAGCTCTCT CTAGCTCTGT  
551 TAAATTGAAG GACCGGCTA ATAGAACAG CATATAAGG AAGACTGAAA  
45 601 ATTATTTCAA AACCTTAAA CGCAGATGAT TTGCGCGCTG CAATACTGAA

651 AGGGACAAAC GGAGATTTGC TTGAAGGGTT TAACGCAGGA CTGTGTAAAA  
 701 CACGACGCTC AGGAAATAC GATGCTATAA AACAGCGGTA TCGTCTCC  
 751 TAA

The PSORT algorithm predicts an inner membrane location (0.452).

- 5 The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 6A. The recombinant protein was used to immunise mice, whose sera were used for FACS analysis (Figure 6B). A his-tagged protein was also expressed.

The cp6900 protein was also identified in the 2D-PAGE experiment (Cpn0604).

- These experiments show that cp6900 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 7

The following *C.pneumoniae* protein (PID 4377033) was expressed <SEQ ID 13; cp7033>:

1 MYNPIGPGPI DEFTERTPPAD LSAQGLEASA ANKSAAEQRI AGAEAPKES  
 51 KTDSEVERNSI LRSAYNALMS LADKLGIASS NSSSTSRSA DVDSTTATAP  
 101 TFPPTTDDY KTOAQTYDT IPTSTSLADI QALVSLQDA VYNIKUTAT  
 151 DEBETAIAAHW ETKNADAVKV GAQITELAKY ASDNQAILDS LGKLTSPULL  
 201 QAALLQSVAN HNKAASLLKE MQDNFVVPKG TPAIAQSLVD QDATATQIE  
 251 KDGNAIRDAY FAGQNASGAV ENAKSNNSIS NIDSAAKAAIA TAKTOTARAO  
 301 KKPPDPSILQ EAEQNVIAQE KDLQNIKPAD GSDVPNPNGIT VOGSKQQGSS  
 351 IGSIRVSMML DDAENETPAI LMSGPRQMIH MFWENPDSQ AAQGEIAAQA  
 401 RAAKAAAGDS AAAALADAKQ ALAALGEAG QQQGINLQD QIASAAVSA  
 451 GVPPAAASSI GSSVQKLYKT SKSTGSDYKT QISAGVDYAK SINDAYGRAR  
 501 NDATRDVINN VSTPALTRGV PRARTEARGP EKTQDALRV ISGNSTRLDG  
 551 VYSGVSLQSS VMQITQSNPO ANNEIRQKL TSAVTKPPQF GYPVQLSND  
 25 601 TQKPIAKLE SLFABGSRFA AEIKALSPET NSLFIQQLV NVGLSVGYSL  
 651 Q\*

The cp7033 nucleotide sequence <SEQ ID 14> is:

1 ATGGTAAATC CTAITGGTCC AGGTCCCTATA GACGAAACAG AACGCACACC  
 51 TCCCGCAGAT CTCTCTGCTC AAGGATTGGA GCGAGTGCA GCAATTAAGA  
 101 GTGCGGAAGC TCAAAAGATA GCAGGTGCGG AAGCTAAGCC TAAAGAAATCT  
 151 AAGACCGATT CTGTAGAGCG ATGGAGCATC TTGGCTTCTG CAGTAAATGC  
 201 TCTCATGAGT CTGCGAGATA AGCTGGGTAT TGCTTCTAT AACAGCTCGT  
 251 CTCTCTACTAG CAGATCTGCA GAGCTGGACT CAGCGCACAG GACCCACCT  
 301 ACGCTCTCTC CACCGACGCA CAGCTGACTT CAACATCACT AGCTGACAG  
 351 TTACGATATC ATCTTACTCT CACATCACTT TAAGAGATAC AGCGCTACT  
 401 TGCTGAGCCT CCAAGATGCT GTCTCACTAA TAAGAGATAC AGCGCTACT  
 451 GATGAGGAAA CCGCAATCGC TCGGAGTGGG GAACTAAGA GTCCGATGC  
 501 AGTTAAAGTT GCGCGGCAAA TTACAGAAAT AGCGAAATAT GCTTCGATA  
 551 ACCAAGCGAT TCTTGTCTCT TTAGGTAAAC TGACTTCTCT GACCTCTATA  
 601 CAGGCTGCTC TTCTCCAAAT TGTAGCAAAAC AATAACAAAG CAGCTGAGCT  
 651 TCTTAAAGAG ATGCAAGATA ACCAGTAGT CCGAGGAAA ACGCTGCAA  
 701 TTGCTCTAAT TTATGTTGAT CAGACAGATG CTACAGCGAC ACAGATAGAG  
 751 AAAGATGGAA ATGCGATTAG GATGCAATTA TTTCAGAGC AAGACGCTAG  
 801 TGAGCTGTGA GAAATGCTA AATCTAATA CAGTATAAG AACATAGATT  
 851 CAGCTAAGAC AGCAATGCTT ACTGCTAAGA CACAATATCG TGAAGCTCAG  
 901 AAAAAGTTC CCGACTCTCC AATCTTCAA GAAGCGGAC AATGTGTAAT  
 951 ACAGGCTGAG AAGATCTTAA AAAATATCAA ACTCTCAGAT GTTCTGATG  
 1001 TTCCAAATCC AGCAACTACA GTTGGAGGCT CCAAGCACAA AGGAAGTAGT  
 1051 ATTGGTAGTA TTCTGTGTTT CATGCTGTGA GATGATGCTG AAAATGAGC  
 1101 CCGCTTCAAT TTGANGCTG GGTTCGTGCA GATGATCTAC ATGTCTCAAT  
 1151 CGGAATAATC TGATTCTCAA GCTGCCCAAC AGGAGCTGCG AGCAACAGCT  
 1201 AGAGCAGCGA AAGCGCTGG AGATGACAGT GCTGTCGAC CGCTGGCAG  
 1251 TGCTCAGAAA GCTTTAGAAG CGGCTCTAGT TAAAGCTGCG CACAAACAGG  
 1301 GCATACCTCA TGTCTTAGGA CAGATCGCTT CTGCTGCTGT TGTGACGCA  
 1351 GGAATTCCTC CCGCTGCGAG AAGTCTTATA GGTCATCTG TAAACAGCT  
 1401 TTACAAAGACC TCAAAATCTA CAGGTCTGAG TTAATAAACA CAGATATCAG

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1451 CAGGTATGA TGCTTACAAA TCCATCAATG ATGCCTATGG TAGGGCACGA  
 1501 AATGATGCGA CTCGTGATGT GATAACAAT GTAGTACCC CCGCTCTCAC  
 1551 ACGATCCGTT CCTAGAGCAC GAACAGAAC TCGAGGACCA GAAAAACAG  
 1601 ATCAAGCCCT CGTAGGGTG ATTCTGGCA ATAGCAGAAC TCTTGAGATG  
 1651 GTCATAGATC AAGTTTCGGC ACTACAATCT GTAATGCAGA TCATCCAGTC  
 1701 GAATCCTCAA GCGAATAATG AGGAGATCAG ACARAAGCTT ACATCGGAGC  
 1751 TGACAAAGCC TCCACAGTTT GGTATCCTT ATGTGCAACT TTCTAATGAC  
 1801 TCTACACAGA AGTTCTATAGC TAAATTAGAA AGTTTOTTGT CTGAAGGATC  
 1851 TAGGACAGCA GCTGAAATAA AAGCACTTTC CTTTGAAGAC AACTCCTTGT  
 1901 TTATTACAGA GGTGCTGGTC AATATCGGCT CTCTATAATC TGGTATATCT  
 1951 CAATAA

The PSORT algorithm predicts a cytoplasmic location (0.272).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 7A. A his-tagged protein was also expressed. The recombinant proteins were used to immunise mice, whose sera were used for FACS (Figure 7B) and Western blot (7C) analyses.

The cp0733 protein was also identified in the 2D-PAGE experiment (Cpn0728) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp0733 a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

## 20 Example 8

The following *C. pneumoniae* protein (PID 6172321) was expressed <SEQ ID 15; cp0017>:

1 MGIKGTGIIV WVDDATAKTK NATLWTKWKG YKPNPERQGP LVPNSLWGSF  
 51 VDVRISQSLM DRSTSSLSST TNLWVSGIAD FLHEDQKQNG RSVRHSAGY  
 101 ALGGGFFFTAS ENFFNFAPCQ LFGYDKDHLV AKNHTHVYAG AMSYRHLSGS  
 151 KTLAKLISGN SDSLFPVFNA RFAYGTDNN MTKRYTGVSP VKGSWGNDAF  
 201 GIECGALPVP VASGRSSWVD THTPFLNLEM IYAHQNDPKE NGTEGRSPQS  
 251 EDLEFNLAIVP GIKPEKFSIDK STYDLSLIAYV PDVIRNDPGC TTTLMVSGDS  
 301 WSTCGTSLSR QALLVRAGNH HAFASNFEPV SQFEVELRGS SRSYADLGLG  
 351 RFGF\*

30 The cp0017 nucleotide sequence <SEQ ID 16> is:

1 ATGGGTATCA AGGGAACCTG AATAMTIGTT TGGGTCGACG ATGCAACTGC  
 51 AAAAAACAAA AATGCTACCT TAACCTGGAC TAAACACGGA TACAAGCCGA  
 101 ATCCAGAACG TCAGGGAACCT TTGGTTCCTA ATAGCCTGTG GGGTCTTTCT  
 151 GTGCAATGCC GCTCCATTCG AGGCCTCATG GACCGGAGCA CAACTTCGTT  
 201 ATCTTCGTCA ACNAAATTTGT GGGTATCAGG AATCGCGGAG TTTTTCATG  
 251 AAGATCAGAA AGGAACCAA CGTAGTTATC GTCAATCTAG CGCGGGTTAT  
 301 GCAITAGGAG GAGGATTCCT CACGGCTTCT GAARATTTCT TTAAATTTTG  
 351 TTTTTCGTAG CTTTITGGCT ACGACAAGGA CCACTCTGTG GCTAAGAAC  
 401 ATACCCATGT ATATGACGGG GCATAGATTT ACAGCAACCT CGGAGAGTCT  
 451 AAGACCCCTG CTAAGATTTT CTCAGGAAAT TCTGATCTCC TACCTTTTGT  
 501 CTTCAATGCT CGGTTTGTCT ATGCCATATC CGACANTAAC ATGACCACAA  
 551 AGTACACTGG CTAATCTCCT GTTAAAGGGA CCGTGGGAAA TGATGCCTTC  
 601 GGTATAGATG GTGGAGGAGC TATCCCGGTA GTTCTTCAG GACCTCGGTC  
 651 TTGGGTGGAT ACCCCACAGC CATTCTCTAA CTTAGAGATG ATCTATGCAC  
 701 ATCAGAAATGA CTTTAAAGAA AACGGCACAG AAGCGCGTTC TTTCCAAAGT  
 751 GAAGACCTCT TCAATCTAGC GGTCTCTGTA GGGATATAAT TTGAGAAAT  
 801 CTCGATTAAG TCTACGTATG ATCTCTCCAT AGCTTACGTT CCGGATGTGA  
 851 TTCCGAATGA TCCAGGCTGC ACGACAACCT TTATGGTTTC TGGGGATCTC  
 901 TGGTCGACAT GTGGTACAGC CTTGTCTAGA CAGACTCTTC TTGTACGTGC  
 951 TGGAAATCAT CATGCCCTTG CTTCAAACTT TGAAGTTTTC AGTCAGTTTG  
 1001 AAGTCAGATT GCGAGGTCTC TCTCGTAGCT ATGCTATCTCA TCTTGAGAGGA  
 1051 AGATTCCGAT TTATA

This sequence is frame-shifted with respect to cp0016.

The PSORT algorithm predicts a cytoplasmic location (0.075).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 8A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 8B) and for FACS analysis (Figure 8C). A his-tagged protein was also expressed.

- 5 This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp0017 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 9

- 10 The following *C. pneumoniae* protein (PID 6172315) was expressed <SEQ ID 17; cp0014>:

```

1  MKSSFQKVFV STFAIFPLSM IATETVLDSS ASFDGNKNGN FSVRESQEDA
51  GTTYLFKGNV TLENIPGTGT AITKSCFNNT KGDLTPTGNG NSLLFQTVDA
101 GTVAGAAVNS SVVDKSTTFI GFSSLSFIAS PGSSITTKGK AVSCSTGSL
151 LTKMSVCSSA KTFQRIMAVL SPQKLFH*
```

- 15 The cp0014 nucleotide sequence <SEQ ID 18> is:

```

1  ATGAAGTCTT CTTTCCCAA GTTTGTATTT TCTACATTG CTATTTCCC
51  TTTGCTATNG ATTGCTACCG AGACAGTTT GGATCAAGT GCGAGTTTCG
101 ATGGGAATAA AAATGGAATP TTTTCAGTTT GTGAGATCA GGAAGATGCT
151 GGAACATACC ACCTATTAAA GGGAAATGTC ACTCTAGAAA ATATTCTGG
201 AACAGGCACA GCAATCACAA AAGCTGTTT TAACACACT AAGGGCGATT
251 TGACTTTCAC AGGTAACGGG AACTCTCTAT TGTCCAAAC GGTGGATGCA
301 GGGACTGTAG CAGGGCGTGC TGTTAACAGC AGCGTGGTAG ATAAATCTAC
351 CACGTTTATA GGGTTTCTT CGCTATCTTT TATTGCGTCT CCTGGAAGTT
401 CGATAACTAC CGGCAARGGA GCCGTTAGCT GCTCTACGGG TAGCTTGAGT
451 TTGACAAAAA TGTCAATTTG CTCTTCAGCA AAACTTTTTC AACGGATAAT
501 GCGCGTGCTA TCACCGCAA AACTCTTTCA TTA
```

This protein is frame-shifted with respect to cp0015.

The PSORT algorithm predicts an inner membrane location (0.047).

The protein was expressed in *E. coli* and purified as a his-tag product, as shown in Figure 9A. A GST-fusion was also expressed. The recombinant proteins were used to immunise mice, whose sera were used in an immunoassay (Figure 9B) and for FACS analysis (Figure 9C).

- 30 This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

- These experiments suggest that cp0014 is a useful immunogen. These properties are not evident from the sequence alone.

### Example 10

The following *C. pneumoniae* protein (PID 6172317) was expressed <SEQ ID 19; cp0015>:

- ```

1  MSALFSEMTS SKKGGAIQTS DALTITGNQG EVSFDNTSTS DSGAAIFTEA
51  SVTISNNAKV SFIDNKVTGA SSSTTGDMGQ GAICAVKTSST DTKVTLTGNQ
101 MLLFSNNTST TAGGAIYVKR LELASGGLTL FGRNSVNGGT APKGGALALE
151 DSGELSLGAD SGDIVPLGNT VTSTTPGTWNR SSDLTGSATK MTAALSAAGR
```

-50-

201 AIYFYDPITT GSSTTVTDVL KVNETPADSA LQYTGNIIFT GKLSSETEAA  
 251 DSKNLTSLKL QPVTLSSGTL SLKHGVLQOT QAFVQQADSR LEMDVGTLE  
 301 PADTSTINNL VINISLIDGA KKAKIETKAT SKNLTLSKFTI TLLDPTGTFF  
 351 ENHSLRNPQS YDILELKSAG TVTSTAVTDP PINGEKGVHY YQGIWGPVIV  
 401 GTGASTTATP NWTKTGYIPN PERIGSLVPN SLWNAFIDIS SLHYLMETAN  
 451 EGLQGDRAFW CAGLSNPFHK DSTRTRRGRF HSLSGVTVGG NLHSTSKDLK  
 501 SAAFCQLFGR DRDYFVAKNQ GTYVGGTLYI QHNETYISLP CKLRPCSLSY  
 551 VPTEIPVLFS GNLSYIHTND DLKTYTTPY TVKGSWNDS FALEFGGRAP  
 601 ICLDESALFE QYMFPMKLFV VYAHQGEFKE QGTEAREFSS SRLVNLALPI  
 651 GTRFDKESDC DATYNLTIG YTVDLVRNPF DCTTLRLISG DSWKTFGLNL  
 701 ARQALVLRAG MHFCYNSNPE AFSQFSFEIR GSRRNVNDEL GAKYQF\*

This sequence is frame-shifted with respect to cp0014.

The cp0015 nucleotide sequence <SEQ ID 20> is:

1 ATGTGACGTC TGTTTTCTGA AATACCTCC TCAAAGAAAG GCGGAGCCAT  
 51 TCAGACTTCC GATGCCCTTA CCATTACTGG AAACCAAGGG GAACTCTCTT  
 101 TTTCTGACAA TACTTCTTCG GATTCTGGAG CTGCAATTTT TACAGAGGCC  
 151 TCGGTGACTA TTTCTAATAA TGTCTAAAGT TCCTTTATTG ACAATAAGGT  
 201 CACAGGAGCG AGCTCCTCAA CAACGGGGGA TATGTCAGGA GGTGCTATCT  
 251 GTGCTTATAA AACTAGTACA GATCTAAGG TCACCTCAGC TGGAAATCAG  
 301 ATGTTACTCT TCAGCAACAA TACATCGACA ACACGGGGAG GAGCATCTCA  
 351 TGTGAAAAAG CTCGAACTGG CTTCGGGAGG ACTTACCCTA TTCAGTAGAA  
 401 ATAGTGTCAA TGGAGGTACA GCTCCTAAAG GTGGAGCCAT AGCTATCGAA  
 451 GATAGTGGGG AATTGAGTTT ATCCGGCGAT AGTGGTGACA TTGTCTTTT  
 501 AGGGAATACA GTCACTCTTA CTACTCTCGG GACGAATAGA AGTAGTATCG  
 551 ACTTAGGAAC GAGTGCAGAG ATGACAGCTT TCGGTCTCTC TGCTGTAGTA  
 601 GCCATCTACT TCTATGATCC CATACCTACA GGATCATCCA CAACAGTTAC  
 651 AGATGTCTTA AAGTTAATG AGACTCCGGC AGATTCTGCA CTACAATATA  
 701 CAGGGAACAT CATCTTCACA GGAGAAAAGT TATCAGAGAC AGAGGCCCGCA  
 751 GATCTTAAAA ACTCTATCTC GAAGCTACTA CAGCCTGTAA CTCTTTCAGG  
 801 AGGTACTCTA TCTTTAAAA ATGAGGTGAC TCTCGAGACT CAGGCATCTA  
 851 CTCACACAGC AGATTCTCGT CTCGAAATGG ACGTAGSAAC TACTCTAGAA  
 901 CCTGCTGATA CTAGACCATC AAACAATTTG GTCAATTAACA TCGATTCTAT  
 951 AGACGCTGCA AAGAAGGCAG AAATAGAAAC CAAGCTACG TCAAAAAATC  
 1001 TGACTTTATC TGGAAACATC ACTTTATTGG ACCCGACGGG CAGCTTTTAT  
 1051 GAAAAATCAT GTTTAAGAAA TCTCAGTCC TACGACTCTA TAGAGCTCAA  
 1101 AGCTTCTGGA ACTGTAAACA GCACCGCAGT GACTCCAGAT CCTATAATGG  
 1151 GTAGAAATTA CCATTACGCG TATCAGGGAA CTTGGGGCCC AATTGTTTGG  
 1201 GGGACAGGGG CTTCTACGAC TGCACACCTC AACTGGACTA AAACCTGGCTA  
 1251 TATTCCTAAT CCCGAGCGTA TCGGCTCITT AGTCCCTAAT AGCTTATGGA  
 1301 ATGCAATTAT AGATATTAGC TCTCTCCATT ATCTTATGGA GACTGCACAA  
 1351 GAGGGCTTGC AGGAGAGCCG TGCCTTTTGG TGTGTGTGAT TATCTAACTT  
 1401 CTTCCATAAG GATAGTACAA AAACACGACG CGGGTTTCGC CATTTGAAGT  
 1451 GCGGTATATG CATAGGAGGA AACCTACATA CTTGTTTACA TAAGATCTT  
 1501 AGTGCTGCAT TTTGTCAGCT CTTTGGAGGA GATAGAGACT ACTTGTATGC  
 1551 TAGAATACAA GGTACAGTCT ACGGAGAAC TCTCTATTAC CAGCAACAGC  
 1601 AAACCTATAT CTCTCTTCTC TGCACACCTC GGCCPTGTCT GTTGCTCTAT  
 1651 GTTCCCAAG AGATTCTGTGT TCTCTTTTCA GGAAACCTTA GCTACCCCA  
 1701 TACGGATAAC GATCTGAAAA CCAAGATATC AACATATCTCT ACTGTTAAG  
 1751 GAGCTCTGGG GAATGATAGT TCTGCTTTAG AATTCCGTCG AAGAGCTCOG  
 1801 ATTTGCTTAG ATGAAAGTGC TCTATTAGAG CAGTACATGC CCTCTATGAA  
 1851 ATTGCAAGTT GTCATGACAC ATCAGGAAGG TTTTAAAGAA CAGGGAACAG  
 1901 AAGCTCTGTA ATTGGAAGT AGCGGCTCTG TGAATCTCTG CTTCACTATC  
 1951 GGGATCCGAT TTGATAAGGA ATCAGACTGC CAAGATGCAA GGTCAAACT  
 2001 AACTCTGGGT TATACTGTGG GATTGTGTGG TAGTACACCC GACTGTAGGA  
 2051 CACACTCGCG AATTAGCGGT GATCTCTGGA AAACCTTCGG TACGAATTGG  
 2101 GCAAGACAGG CTTTAGTCTT TGTGTGAGG AACCAATTTT GCTTAACTC  
 2151 AATTTTGAAG CCTTTTAGCC AATTTCTTT TGAATGCGGT GGGTACTCTC  
 2201 GCAATTAACA TGTAGACTTA GGAGCAAAAT ACCAATCTTA A

The PSORT algorithm predicts a cytoplasmic location (0.274).

60 The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 10A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 10B) and for FACS analysis. A his-tagged protein was also expressed.



These experiments show that cp0015 is a useful immunogen. These properties are not evident from the sequence alone.

### Example 11

The following *C.pneumoniae* protein (PID 6172325) was expressed <SEQ ID 21; cp0019>:

```

5      1  LQDSQDYSFV  KLSFGAGGTI  ITQDASQKPL  EVAPSRPHYG  YQGHWNVQVI
51     51  PGOTQTQPSQA  NLEWVRGTGVL  FNFRQGSGLV  PNLWVGSPVD  QRAIQEIMVN
101    101  SSQILCQERG  VWGAGIANFL  HRDKINEHGY  RSHGVGYLVG  VGTAFSDAT
151    151  INAAFCQLFS  RDKDYVYSKN  HGTSYSGVVF  LEDTLFERSP  QGFYDSSSE
201    201  ACCNQVVTD  MQLSYSHRNN  DMKTKYTTYF  EAQGSWANDV  FGLEFGATTY
10     251  YYPNSTFLFD  YYSFPLRLQC  TYAHQEDPKE  TGGEVRHPTS  GDLENLAVPI
301    301  GVKFERFSDC  KRGSVELTLA  YVPDVIRKDP  KSTATLASGA  TWSTHGNLNL
351    351  RQGLQLRLGN  HCLINPGIEV  FSBGAIELRG  SSRNYNINLG  GKYPF*

```

This sequence is frame-shifted with respect to cp0018.

The cp0019 nucleotide sequence <SEQ ID 22> is:

```

15     1  TTGCAAGACT  CTCAAGACTA  TAGCTTTGTA  AAGTTATCTC  CAGGAGCGGG
51     51  AAGGACTATA  ACTACTCAAG  ATCGCTTCTA  GAAGCCTCTT  GAAGTAGCTC
101    101  CTTCTAGACC  ACATATAGGC  TATCAAGGAC  ATTGGAATGT  GCAAGCCTAC
151    151  CCAGGAACGG  GAACTCAAAC  GAGCCAGGCA  AATTAGAAAT  GGGTGCAGAC
201    201  AGGATACCTT  CGAATCCCG  AACGCCAAGG  ATCTTAGATT  CCCAATAGCC
20     251  TGTGGGGTTC  TTTTGTGTAT  CAGCGTGCTA  TCCAAGAAAT  CATGTAATAT
301    301  AGTAGCCAAA  TCTTATGTCA  GGAACGGGGA  GTCTGGGGAG  CTGGAATTGC
351    351  TAAATTCCCTA  CATAGAGATA  AAATTAATGA  GCACGGCTAT  CGCCATAGCC
401    401  GTGTCCGTTA  TCTTTGTGGA  GTTGGCACTC  ATGCITTTTC  TGATGCTAGC
451    451  ATAAATGCGG  CTTTTTGCCA  GCTCTTCAGT  AGAGATAAAG  ACTACGTAGT
25     501  ATCCAAAAAT  CATGGAACCT  GCTACTCAGG  GGTCTGATTT  CTTGAGGATA
551    551  CCCTAGAGTT  TAGAAGTCCA  CAGGAGTCTC  ATACTGATAG  CTCTCCAGAA
601    601  GCTTCCTGTA  ACCAAGTCGT  CACTATAGAT  ATGCAGTTGT  CTTACGCCCA
651    651  TAGAATAAAT  GATATGAAAA  CCAATATACAC  GACATATCCA  GAAGCTCAGG
701    701  GATCTTGGGC  AAATGATGTT  TTTGGTCTTG  AGTTTGGAGC  GACTACATAC
30     751  TACTACCCCTA  ACAGTACTTT  TTTATTGTAT  TACTACTCTC  CGTTTCTCAG
801    801  GCTGCAGTGC  ACCTATGCTC  ACCAGGAGA  CTTCAAAGAG  ACAGGAGGTG
851    851  AGGTTCTGTC  CTTTACTAGC  GAGATCTTGT  TCAATTTAGC  AGTTCTTATT
901    901  GGCCTGAAGT  TTGAGAGATT  TTCAGACTGT  AAAAGGGGAT  CTTATGAATC
951    951  TACCCTTGGT  CATGTTCCTG  ATCTGATTCG  CAAAGATCCC  AAGAGCAGCG
35     1001  CAACATTGGC  TAGTGGAGCT  ACGTGGAGCA  CCCACGGAAA  CAATCTCTCC
1051   1051  AGACAAGGAT  TACAAGTCTG  TTTAGGGAAC  CACTGCTCTCA  TAAATCTCTG
1101   1101  AATTCAGGTT  TTCAGTCACG  GAGCTATTGA  ATTCGGGGGA  TCCCTCTGTA
1151   1151  ATTATACAT  CAATCTCGGG  GGTAAATACC  GATTTTAA

```

The PSORT algorithm predicts a cytoplasmic location (0.189).

40 The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 11A. This protein was used to immunise mice, whose sera were used in a Western blot (Figure 11B) and an immunoblot assay (Figure 11C). A his-tagged protein was also expressed.

These experiments show that cp0019 is a useful immunogen. These properties are not evident from the sequence alone.

### Example 12

The following *C.pneumoniae* protein (PID 4376466) was expressed <SEQ ID 23; cp6466>:

```

50     1  MNKISVOICL  TILLSLSVVL  QGCKESSHSS  TSGRLAINI  RDEPRSLDPR
51     51  QVRLSEISL  VKHIYBGLVQ  ENNLSGNIEP  ALAEDYSLSS  DGLTYTFKLK
101    101  SAFPWSNDPL  TAEDFIESWK  QVATQEVSGI  YAFALNPITN  VRKIQEGLHS
151    151  IDHPGVHSPN  ESTLVVTLES  PTSHFLKLLA  LFVFFPVHKS  QKTLQSKSLP
201    201  TASGAFYFKN  IKQKQWIKLS  KNEPHYNQSQ  VETKTTITHP  IPDANTAAKL

```

-52-

251 FNQGRKLNWQG PFWGERIPQRE TLSNLSQSEGH LHSFDVAGTS WLTPNINKFP  
 301 LNNMKLREAL ASALDKRALV STIFLGRAKT ADHLPTNIH SYVPHQKQEM  
 351 AQRQAYAKKL PKEALEBLQI TAKDLHLHLN IPFVSSSSAS LVLQLRLSQEM  
 401 KESLGFPAIP VGKEFALLQA DLSGNGFLA TGGWFPADPAD PMAPLTLFAY  
 451 PSGVPPYAIN HKDFLEILQN IEQEQDQKR SELVSAQSLY LETPHILIEPI  
 501 YHDAFQFAMI KKLNLGVSP TGVVDFRYAK EN\*

A predicted signal peptide is highlighted.

The cp6466 nucleotide sequence <SEQ ID 24> is:

1 ATGCGCAAGA TATCAGTGGG AATCTGTATC ACCATCTCTC TTAGCCTCTC  
 51 CGTAGTCCCTC CAAGGCTGCA AGGAGTCCAG TCACTCTCTCT ACATCTCGGG  
 101 GAGAACTCCG TATTAATATA AGAGATGAAC CCGGTCTCTT AGATCCAAGA  
 151 CAAGTCCGAC TCTCTTCAGA AATCAGCCTT GTCAACATA TCTATGAGGG  
 201 ATTAGTTCAA GAAATAATC TTTCAGGAA TATAGACCT GCTCTTGACG  
 251 AAGACTACTC TCTTTCCTCG GACGGACTCA CTATACTTT TAAACTGAAA  
 301 TCAGCTTTT TTGGAGTAATG CGACCCCTTA ACAGCTGAAG ACTTTATAGA  
 351 ATCTTGGAAA CAAGTAGCTA CTCAGAAGT CTCAGGAATC TATGCTTTTG  
 401 CCTGTAAATC AATTAAAAAT GTACGAAAGA TCCAAAGAGG ACACCTCTCC  
 451 ATAGACCAAT TTGGAGTGCA CTCCTCTAAT GAATCTACAC TTGTGTTTAC  
 501 CCTGGAATCC CCAACCTCGC ATTTCTTAAA ACTTTTAGCT CTTCCAGTCT  
 551 TTTTCCCGCT TCATAAATCT CAAAGAACCC TGCAATCCAA ATCTCTACCT  
 601 ATAGCAAGCG GAGCTTTCTA TCTTAAAAAT ATCAACAAA AACAAATGGAT  
 651 AAAAATCTCA AAAAACCTCT ACTACTATA TCAAGTCAG GTGGAAACTA  
 701 AAACGATTAC GATTCACCTC ATTCCCGATG CAAACACAGC AGCAAACTA  
 751 TTTAATCAGG GAAAACTCAA TTGGCAAGGA CCTCCTTGGG GAGAAAGCAT  
 801 TCTCTAAGAA ACCCTATCCA ATTTACAGTC TAAGGGGCAC TTCACTCTT  
 851 TTGATGTGCG AGGAACCTCA TGGCTCACT TCAATATCAA TAAATTCGCC  
 901 CTCACAAATA TGAAGCTTAG AGAAGCCTTA GCATCAGCCT TAGATAAGGA  
 951 AGCTCTTGTC TCAACTATAT TCTTAGGCG TGCAAAACTC GCCGATCAT  
 1001 TCTTACCTAC AATAATTCAT AGCTATCCCG AACATCAAAA ACAGAGATG  
 1051 GCACAACGCC AAGCTTAAGC TAAAAAATCT TTTAAAGAGC CTTTGAAGA  
 1101 ACTCCAATCT ACTGCTAAG ATCTCGAACA TCTTATCTT ATCTTTCCCG  
 1151 TTTCTCTGTC AGCAAGTTCT TTAATAGTCC AACCTTATAC AGAACAAGTG  
 1201 AAAGAAAGTT TAGGGTTGCG TATCCCTATT TGCGGAAAGG AATTGTCTCT  
 1251 TCTCTAAGCA GACCTATCTT CAGGGAACCT CTCTTTAGCT ACAGGAGGAT  
 1301 GGTTCGACGA CTTTCTGAT CCTATGGCAT TTCTAAGCAT CTTTGTCTAT  
 1351 CCATCAGGAG TTCTCTCTTA TGCAATCAAC CATAAGGAT TCTTGAATAT  
 1401 TCTACAAAC ATAGAACAAG AGCAAGATCA CCAAAACGCG TCGGAATTAG  
 1451 TGTCGAAGC TTTCTCTTAC CTAGAGACCT TTCAATATAT TGAGCGGAT  
 1501 TACCAGACG CATTTCAATT TGCTATGAAT AAAAATCTT CTAACTAGG  
 40 1551 AGTCTACCA ACAGGAGTTG TGGACTTCCG TTATGCTAAG GAAAAATTAG

The PSORT algorithm predicts that the protein is an outer membrane lipoprotein (0.790).

The protein was expressed in *E. coli* and purified both as a GST-fusion product and a His-tag fusion product. Purification of the protein as a GST-fusion product is shown in Figure 12A. The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 12B and 12C). FACS analysis was also performed.

These experiments show that cp6466 is a useful immunogen. These properties are not evident from the sequence alone.

### Example 13

The following *C. pneumoniae* protein (PID 4376468) was expressed <SEQ ID 25; cp6468>:

1 MFSRWITLFL LFLSLTGCS YSKHKQSLI IPIHDPVAF SPEQAKRAMD  
 51 LSLIAQLLFDG LTRETHRESN DLELAIASRY TVSEDFCSVT FFIKDSALMS  
 101 DSTPTTSED I RNAWEYAQEN SPHIQIFQGL NFSTPSSNAI TIHLDSPNFD  
 151 FPKLLAFPAF ALFKPENFKL FSGPYTLVEY FPGHNHLKK NPNYDYHCV  
 201 SINSIKLLII PDITYATHLL NRGKVDNVGQ PWHQGIWEL HKQSYHYTT  
 55 251 YVVEGAFWLC LNTKSPHLND LQNRHRLAET IDKRSIIEBA LQGQQQFABT

```

301 LSRGAPQPNQ YKKQKPLTPQ EKLVLTYPSD ILRCQRIARI LKEQWKAAGI
351 DLILLEGLEYH LFNKRRKVDQ YAIATQTGVA YYPGANLISE EDKLLQNFBFI
401 IPIYLYSDY LTQDFIBGVI YNAGSAVDLK YTYFF*

```

A predicted signal peptide is highlighted.

5 The cp6468 nucleotide sequence <SEQ ID 26> is:

```

1 ATGTTTTCAC GATGGATCAC CCTCTTTTTA TTATTCATTA GCCTTACTGG
51 ATGCTCCTCC TACTCTTCAA AACATAAACA ATCTTTTAATT ATTCOCATAC
101 ATGACGACCC TGATGCTTTT TCTCTGAAC AAGCAAAGAC GGCCATGGAC
151 CTTTCTATTG CCCAACTTCT TTTTGATGGT CTGACTAGAG AAACATCATCG
10 201 CGAATCCAAAT GATTTGGAAAT TAGCGATTGC CAGTCGCTAT ACAGTCTCTG
251 AAGACTTTTG CTCTTATACG TTCTTTATCA AAGACAGCGC TTTATGGAGC
301 GACGGAAACAC CAATCACTCT CGAAGATATC CGTAACGCTT GGGAGTATGC
351 ACAGGAGAAC TCTCCCCACA TACAGATCTT CCAAGGACTT AACTTCTCAA
401 CTCCTTCAATC AAATGCAATT ACGATTTCAT TCGACTCGCC CAACCCCGAT
15 451 TTTCTTAAGC TTCTTGCTTT TCTTGCATTT GCTATCTTTA AACCAGAAAA
501 CCCGAAGCTC TTTAGCGGTC CGTATACTCT TGTAGAGTAT TTCCAGGGC
551 ATAAACATCA TTAAAGAGAA AACCCCTAAT ATTAGCACTA CCACCTGGTC
601 TCCATCAACT CCATCAAACT GCTCATATT CTTGATATAT ATACAGCCAT
651 CCACCTCCTA AACAGAGSCA AGGTGGACTG GGTAGGAGCA CCCTGGCATC
20 701 AAGGGATTCG TTGGGAGCTC CATAAACAAT CGCAATATCA CTACTACACC
751 TATCCTGTAG AAGGTGCTTT CTGGCTTTGT CTAAATACAA AATCCCCACA
801 CTTAAATGAT CTTCAAAACA GACATAGACT CGCTACTTGT ATTGATTAAC
851 GTCTATCATC TGAAGAGGCT TCTCAAGGAA ACCAACACCC AGCGGAAACA
901 CTGTCTCAGG GAGCTCCACA ACCAATCTAA TATAAAGACT AAAGCCCTCT
25 951 AACTCCACAA GAAAACTCTG TGTATACCTA TCCCTCAAGT ATCTTAAGAT
1001 GCCAACCCAT AGCAGAAATC TTTAAGAGAC AATGGAAGC TCCTGGATTA
1051 GATTTAATCT TTGAGAGACT CGAATCAACT CTGTTCCTTA ACACAACGAA
1101 AGTCCAAAGC TACGCCATAG CAACACAGAC TGAAGTTGCT TATTAACGAG
1151 GACCAAACTC AATTTCCTGA GAAGACAAGC TCCTCCAAAA CTTTGAAGAT
30 1201 ATCCCGATCT ACTATCTGAG CTATGACTAT CTCACCTAAG ATTTTATAGA
1251 GGGAGTAATC TATAATGCTT CTGGAGCTGT AGATCTCAA TATACCTATT
1301 TCCCTTAG

```

The PSORT algorithm predicts that this protein is an outer membrane lipoprotein (0.790).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 13A.

35 The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 13B) and for FACS analysis. A his-tagged protein was also expressed.

These experiments show that cp6468 is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 14

40 The following *C. pneumoniae* protein (PID 4376469) was expressed <SEQ ID 27; cp6469>:

```

1 MQMHLKPTL KSLIPNLLFL LLTLSSCSKQ KQPLGKHLV IAMSHDLADL
51 DFRNAYLSRD ASLAKALYBG LTRTFDQGLA LALAEYSTLS KDHRVYTFKL
101 RFSVWSDGTP LTAIDFEKSI KQLYFERFSP SIHTLLGVIK NSSAIHNAQK
151 SLETLGIQAK DDLTLVITLH QPFYFLTLI ARPVFSFVHH TLRBSYKKG
45 201 PPSTYISNGP FVLKKHEHQN YLILEKNPHY YDHSVSKLDR VTLKIIIPDAS
251 TATKLFSKSKS IDWIGSPWSA PISNEDQKVL SQEKILTVSV SSTTLIYNL
301 QKPLIQNKAL RKAIAHAIRD KSLRLVPSG QBAVTLVPFN LSQNLQKBI
351 STEERQTKAR AYPQEAKEPL SEKELABELSI LYPIDSSNS IIAQEIQRQL
401 KDTLGLKIKI QMEYHCFILK KRRQGDFFIA TGGWIAEYVS PVAPLSILGN
50 451 PRDLTQWRNS DYKTEKLKLY LPHAYKENLK RAEMIEEBT PIIPLYHGKY
501 IYAIHPKIQN TFGSLLGHDT LKNIDILS*

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A predicted signal peptide is highlighted.

The cp6469 nucleotide sequence <SEQ ID 28> is:

1 ATGAAGATGC ATAGGCTTAA ACCTACCTTA AAAAGTCTGA TCCCTAATCT  
 51 TCTTTTCTTA TTGCTCACTC TTTCAGAGTG CTCAGAGCAA AAACAAGAAC  
 101 CCTTAGGAAA ACATCTCGTT ATTGCGATGA GCCATGATCT CGCCGACCTA  
 151 GATCCTCGCA ATGCCTATTT AAGCAGAGAT GCTTCCCTAG CAAAGCCCTC  
 5 201 CTATGAAGGA CTGACAAGAG AAATGATCATA AGGATCTATC CTGGTCTCTG  
 251 CAGAAAGTTA TACCCTGTCA AAAGATCATA AGGCTATATC CTTTAACTC  
 301 AGACCTTCTG TGTGAGCGGA TGGCAGTCCA CTCACTGCTT ATGACTTTGA  
 351 AAAATCTATA AAACAACCTG ACTTGAAGA ATTTTCACCT TCCATACATA  
 10 401 CTTTACTCGG CGTGATTAAT AATCTCTCGG CAATCCACAA TGCTCAAAA  
 451 TCTCTGGAAA CTCTTGGGAT ACAGGCAAAA GATGATCTTA CTTTGGTGAT  
 501 TACCCTAGAG CAACCTTTCC CACTACTTCT CACACTTATC GCTCGGCCCG  
 551 TATTCTCCCC TGTTCATCAC ACCCTTAGGG AATCCTATAA GAAAGGAACA  
 601 CCCCCATCCA CATACATCTC CAATGGGCC TTTGTCTTAA AAAAACATGA  
 651 ACACCAAAAC TACTTAATTT TAGAAAAAAA TCCTCACTAC TATGATCATG  
 15 701 AATCAGTAA GTTAGACCGA GTCACTTAA AAATTATCCC AGACGCCCTC  
 751 ACAGCCACGA AACTTTTCAA AAGTAAATCT ATAGATTGGA TTGGCTCACC  
 801 TTGGAGCGCT CGGATATCTA CAGAGACCA AAAAGTTCTC TCCCAAGAAA  
 851 AGATCTTAC CTATCTGTTT TCAGACCA CCCTTCTTAT CTATAACCTG  
 20 901 CAAAAACCTC TAATACAAAA TAAAGCCCTC AGGAAAGCTA TTGCTCATGC  
 951 TATTGATAGA AATCTATCTT TAAGACTCGT GCCTTCAGGA CAAAGAGCTG  
 1001 TAACCTTAGT TCCCCCAAT CTTCACAAC TCAATCTCCA AAAAGAGATC  
 1051 TCACAGAAAG AAGCACAAC AAAAGCCAGA GCATATTTTC AAGAAGCTAA  
 1101 AGAACAACCT TCTGAAAAG AACTCGCAGA ACTCAGCATC CTCTATCCTA  
 1151 TAGATTCTCT GAATCTCTCC ATCATAGGTC AAGAAATCCA AAGACAACCT  
 25 1201 AAGATACCTT TAGGATTGAA AATCAAAATC CAAGGCATGG AGTACCACCTG  
 1251 CTTTTPAAG AAACGTGCTC AAGGAGATT CTTCATAGC ACAGGAGGAT  
 1301 GGAATGCGGA ATACGTAGC CCGGTAGCTC TCCTATCTAT TCTAGGCAAC  
 1351 CCCAGAGACC TCACACATG GAGAAACAGT GATTACGAAA AGACTTTTGA  
 30 1401 GAAACTCTAT CTCCCTCATG CCTACAAGA GAATTTAAAA CGCCGAGAAA  
 1451 TGATAATAGA AGAAGAAACC CCGATTATCC CCCTGTATCA CGGCAAAAT  
 1501 ATTTACGCTA TACATCCATA AATCCAGAAT ACATTCGGAT CTCTCTTAGG  
 1551 CCACACAGAT CTCAAAAATA TCGATATCTT AAGTAG

The PSORT algorithm predicts a periplasmic location (0.934).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 14A.

35 The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 14B) and for FACS analysis. A his-tagged protein was also expressed.

These experiments show that cp6469 is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 15

40 The following *C. pneumoniae* protein (PID 4376602) was expressed <SEQ ID 29; cp6602>:

1 MAASGCTGGL GGTQGVNLAA VEAARAKADA ABVVASQBS EMMMIQQSD  
 51 LTNFAAATRT KKKBEKFTLT ESKKKBAGK ABKKSSTEE KPFDLADKY  
 101 ASGNSEISGQ ELRLGRDAG DDAESPEDIA LVQKILFSAQ LQSTALDLYL  
 151 QTPFPQGGKL KEALIQARNT HTEQFGRTAI GAKNILFASQ EYADQLNVSF  
 45 201 SGLSLSLYLEV TGDTHTCQDL LSLMLQDRYTY QDMAIVSSFL MKGMATELKR  
 251 QQFVFSQAQL QVLMTEBRLN QAVLTSYDYF ESRVETLLDS LKAEGJFES  
 301 DLNFVKAES YHKLIINDFP TAAKVEREV NLIGDWDVSV TGVNLFFES  
 351 LRQTSRLRFS SADKRQLQGA MIANALDAVN INNEHYKAS DPKPFPIS\*

The cp6602 nucleotide sequence <SEQ ID 30> is:

50 1 ATGGCAGCAT CAGGAGGCAC AGGTGGTTTA GGAGGCACCT AGGGTGTCAA  
 51 CCTTCAGAGT GTAGAGCTG CAGCTGCAAA AGCAGATGCA GCAGAAGTTG  
 101 TAGCCAGCCA AGAAGGTTCT GAGATGAACA TGATTTCAAC ATCTCAGGAC  
 151 CTGACAATCT CCGCAGCAGC AACACGCAGC AAAAAAAGG AAGAGAAGTT  
 201 TCAAACTCTA GATCTCTGGA AAAAAGGAGA AGCTGGAAGC GCTGAGAAAA  
 55 251 AATCTGAATC TACAGAAGAG AAGCCTGACA CAGATGTCTG TGATAAGTAT  
 301 GCTTCTGGGA ATTTCTGAAT CTCTGGTCAA GAACTTCGCG GCTGCTGTA  
 351 TGCAATAGGA GACGATGCTT CTCCAGAGA CATTCCTGCT CTGTOTACAG

-55-

401 AGAAAAATTAA AGACCCAGCT CTGCAATCCA CAGCTTTGGA CTACCTGGTT  
 451 CAAAACGACTC CACCTCCCA AGSTAATTA AAAGAAGCGC TTATCCAAGC  
 501 AAGGAATACT CATACGGAGC AATTCGGAGC AACTGCTATT GGTGCGAAAA  
 551 ACATCTTATT TGCTCTTCAA GAATATGCG AGCAACTGAA TGTTTCTCCT  
 601 TCAGGGCTTC GCTCTTTGTA CTTAGAGTGC ACTGGAGACA CACATACCTG  
 651 TGATCAGCTA CTTTCTATGC TTCAAGACCG CTAATCCTAC CAAGATATGG  
 701 CTATTGTCAG CTCTCTTCTA ATGAAGGAA TGGCAACAGA ATTAATAAGG  
 751 CAGGGTCCCT ACGTACCCAG TGGCGAACCTA CAACTTCTCA TGACAGAAAC  
 801 TCGTAAACCTG CAAGCAGTTC TTACTTCGTA CGATTACTTT GAAAGTCGCG  
 851 TTCTCTATT TTCTCGATAG TTAAGAAGCTG AGGGAATCCA AACTCTCTCT  
 901 GATCTAAACT TTGTGAAGGT AGCTGAGTCC TACCATAAAA TCATTAAACGA  
 951 TAAGTTCCTCA ACAGCATCTA AAGTAGAAGC AGAAGTCGCG AATCTCATAG  
 1001 GAGACGATGT TGATTCTGTG ACCGGTGTCT TGAACCTATT CTTTCTGCT  
 1051 TTACGTCAAA CGTCGTACAG CTTTCTCTCT TCAGCAGACA AAGCTCAGCA  
 1101 ATTAGGAGCT ATGATTGCTA ATGCTTTAGA TGCTGTAAAT ATAAACAATG  
 1151 AAGATTATCC CAAAGCATCA GACTTCCCTA AACCTATCC TTGCTCAAGA

The PSORT algorithm predicts a cytoplasmic location (0.080).

The protein was expressed in *E. coli* and purified as both a His-tag and a GST-fusion product, as shown in Figure 15A. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 15B) and for FACS analysis (Figure 15C).

The cp6602 protein was also identified in the 2D-PAGE experiment (Cpn0324).

These experiments show that cp6602 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 16

The following *C. pneumoniae* protein (PID 4376727) was expressed <SEQ ID 31; cp6727>:

1 MKYSLFWLLT SSALVFSLHP LMAANTDLSS SDNYEMSSSG SAAFTAKRTS  
 51 DASGTTYTIT SDVSITNVSA ITPADKSCPT NTGGAISFVG ADHSLVLQTI  
 101 ALTHDGAAIN NTNTALFSFG FSSLLIDSAP ATGTSGGKGA ICVTNTEGGT  
 151 ATPTDNASVT LQKNTSEKDG AAVSAYSIDL AKTTTAAALLD QNTSTKNGGA  
 201 LCSTANTTVQ GNSGTVTFFSS NTATDKGGGI YSKRKBSTLD ANTGVVTFKS  
 251 NTAKTGGAMS SDDNLALTGN TQVLFOENKT TGSAAQANNP EGCGGAICCY  
 301 LATATDKTGL AISQNMESF TSNTTTANGG AIYATKCTLD GNTTTLTDFQN  
 351 TATAGCGGAI YTEDEDFSLK GSTGTVTFT NTAKTGGALY SKGNSSLTGN  
 401 TNLPLPSGKA TGPSSNSAAG ECGCGAILAF IDSQSVSDIT GLSIANNQEV  
 451 SLTSSNAATVS GGAIYATKCT LTNGSLTDF GNTAGTSGGA IYETEDFTLL  
 501 TGSTGTVTFS TMTATGGAL YSKGNSLSDG NTNLPLPSGKA ATGPSSNSAN  
 551 QEGCGGAILS FLESASVSTK KGLWIEDNEN VSLSGNTATV SGGAIIYATKC  
 601 ALHGNTTLLT DGNIAETAGG ALYETEDFT LTGSTGTVTFT STNTAKTAGA  
 651 LHTKGNVTPT KKKALVFSGN SATATATTT DQEGCGGAIL CNI SEDSIAT  
 701 KSLTLTENES LSFINNTAKR SGGGIYAPKC VTSQSSSEINF DGNIAETSGG  
 751 AIYSEMLSTIT ANGPVSFTNN SGGKGGAIYI ADSGELSLRA IDGDIYTFGN  
 801 RATBGTSTPN SIHLGAGAKI TKLAAAPGHT IYFYDFTIME APASGGTIEE  
 851 DIVINPVKAI VPPPPQKNGP IASVPVVPVA PANFWGTIV FSSGKLPSQD  
 901 ASIPANTPTTI LMQKINLAGS NVVLKGBATL QVYSFTQQPD SVFVMDAGTT  
 951 LETTTTNTND GSIDLKNLAV NLDALDGKRM LTIAVNSTSG CLKISIGDLKF  
 1001 HNNBGSFYDN RGLKANLALP FLDLSTSTGT VNLDDFNPIF SSMAPADYGI  
 1051 QGSWTLVFKV GAGGKVLIVA EWALGVTPE PELRATLVFN SMLNATVNIH  
 1101 STQGEIATAM SDAPSHPGIV TGGIVGAPHF DKQKHAAGFA LISGIVVGG  
 1151 SMTPDQVETP AVAPSQLPGR SKDQVVDGIK SQVYAGSLCA QSVYVPLGHS  
 1201 SLRRHVLQKV LPLRLPGLSP VLHGQVSYGR NHHNNTKGLA NHTGKSKWD  
 1251 SHSFAVTEGG SLPLVLDNRY LTHSYSPVKL QVVSVMQKFA QVVAADPRTF  
 1301 DASHLVVYSI PMGLTFKHES AKPPSALLLT LGYAVTAYRD HFHCLTSLTN  
 1351 STNSSTFATN LSRQAPFAEA SGHLKLHLGL DCPAGSGCBL RBSRSRYNAN  
 1401 CGTKYSF\*

A predicted signal peptide is highlighted.

The cp6727 nucleotide sequence <SEQ ID 32> is:

|    |      |             |             |             |             |              |
|----|------|-------------|-------------|-------------|-------------|--------------|
|    | 1    | ATGAATATT   | CTTTACCTTG  | GCTACTTACC  | TCTTCGGGTT  | TAGTTTCTTC   |
|    | 51   | CTCATATCCA  | CTAATGGCTG  | CTACACGGGA  | TCTCTCATCA  | TCCGATAACT   |
| 5  | 101  | ATGAATATGG  | TAGTAGTGGT  | AGCGCMGACT  | TCACTGCTAA  | GGAAACTCTG   |
|    | 151  | GATGCTTCAG  | GAATCACTCA  | CACCTCTCACT | AGCGAGTTAT  | CTATTACGAA   |
|    | 201  | TGTATCTGCA  | ATTACTCTCTG | CAAGTAAAGG  | CTGTTTTCAC  | AACACAGGAG   |
|    | 251  | GAGCAITGAG  | TTTGTGTGGA  | GCTGATCACT  | CATTGGTCTCT | GCAACACATA   |
|    | 301  | CGCTTACGCG  | ATGATGGTGC  | TGCAATTAAC  | AATACCAACA  | CAGCTCTTTC   |
|    | 351  | TTTCTCAGGA  | TTCTCGTCAC  | CTCTTAATCGA | CTCAGCTCCA  | GCAACAGGAA   |
| 10 | 401  | CTTCGGGCGG  | CRAGGGTGCT  | ATTGTTGTGA  | CAAAATACAGA | GGGAGGTACT   |
|    | 451  | CGCACTTTTA  | CTGACAAATGC | CACTGTCAAC  | CTCCAAAAAA  | ATACTTCAGA   |
|    | 501  | AAAAGATGGA  | GCTCGATGTT  | CTGCTTACAG  | CATCGCTCTT  | GCTAAGACTA   |
|    | 551  | CGACAGCAGC  | TCTCTTAGAT  | CMAATACTA   | GCACAAAAAA  | TGGCGGGGCC   |
|    | 601  | CTCTGTAGTA  | CAGCAACAC   | TACAGTCCAA  | GAAATCTCAG  | GAACGGTGAC   |
| 15 | 651  | CTTCTCCTCA  | AAATACTGCTA | CAGATAAAGG  | TGGGGGGATC  | TACTCAAJAG   |
|    | 701  | AAAAGGATAG  | CACGCTAGAT  | GCCAAATCAG  | GAGTCGTAAT  | CTTCAAATCT   |
|    | 751  | AATACTGCAA  | AGACGGGGGG  | CACTTGGAGC  | TCTGATGACA  | ATCTTGCTCT   |
|    | 801  | TACCGGCAAC  | ACTCAAGTAC  | TTTTTCAGGA  | AAATAAAACA  | ACCGGCTCAG   |
|    | 851  | CAGCAGCAGC  | AAATAACCGG  | GAAGGTGTGT  | GTGGGGCAAT  | CTGTTGTATAT  |
| 20 | 901  | CTTGCTTACG  | CAACAGACAA  | AACCTGGATTA | GCCATTTCTC  | AGAATCAAGA   |
|    | 951  | AATGAGCTTC  | ACTAGTAATA  | CAACAACTGC  | GAATGGTGGGA | CGCATCTACG   |
|    | 1001 | CTACTAAATG  | TACTCTGGAT  | GGAAACACAA  | CTCTTACTCT  | CGATCAGAAAT  |
|    | 1051 | ACTCGCAGAG  | CAGGATGTGG  | CGGAGCTATC  | TATACAGAAA  | CTGAAGATTT   |
|    | 1101 | TTCTCTTAAG  | GGAAATCAGG  | GAACCGTACG  | CTTCAGACAA  | AATACAGCAA   |
| 25 | 1151 | AGA CAGGCGG | CGCCTTATAT  | TCTAAAGGAA  | ACAGCTCGCT  | GACTGGAAAT   |
|    | 1201 | ACCAACCTGC  | TCTTTTCAGG  | GAACRAAGCT  | ACGGGCGCGA  | GTAATTTCTTC  |
|    | 1251 | ACGAATCAA   | GAGGGTGGCG  | GTGGGGCAAT  | CCTGAGCTTT  | ATTGATTACG   |
|    | 1301 | GATCCGTAAAG | CGATAAAACA  | GGACTATCGA  | TGCAAAACA   | CCAAGAGTCT   |
|    | 1351 | AGCCTCACTA  | GTAATGCTGC  | AACAGTAAGT  | GGTGTGTGGA  | TCTATGCTAC   |
| 30 | 1401 | CAAAATGACT  | CTAACTGGAA  | ACGGCTCCTC  | GACCTTTGAC  | GGCAATACTCTG |
|    | 1451 | CTGGAATTTT  | AGGAGGGGCG  | ATCTATACAG  | AACATGAGAA  | TTTTTACTCTT  |
|    | 1501 | ACAGGAAGTA  | CAGGAACCGT  | GACCTTCAGG  | ACAAATACAG  | CAAGACAGAG   |
|    | 1551 | CGGCGCCTTA  | TATTTCTAAG  | GCAACAACCT  | TCGTCTGGT   | AATACCAACC   |
| 35 | 1601 | TGCTCTTTTC  | AGGGAACAAA  | GCTACGGGCG  | CGAGTAATCT  | TTTCAAGAAAT  |
|    | 1651 | CAAGAGGGTT  | CGGTTGGGCG  | AATCCTATCG  | TTTCTTGAAGT | CAGCATCTGT   |
|    | 1701 | AAATACTAAA  | AAAGGACTCT  | GGATTGAAGA  | TACGGAAGAA  | GTGAGTCTCT   |
|    | 1751 | CTGGTAAATC  | TGCAACAGTA  | AGTGGCGGTG  | CGATCTATGC  | GACCAAGTGT   |
|    | 1801 | GCTCTGCATG  | GAACAACGAC  | TCTTACTCTT  | GATGGCAATA  | CTGCCGAAC    |
|    | 1851 | TGCAAGGAGGA | GCGATCTATA  | CAGAAACCGA  | AGATTTTACT  | CTTACGGGAA   |
| 40 | 1901 | GTACGGGAAAC | CGTACCTTTC  | AGCAACAAAT  | CAGCAAAAGAC | AGCAGGGGCT   |
|    | 1951 | CTACATACTA  | AAGGAATATC  | TTCTTTTACC  | AAAAATAGGG  | CTCTTGTATT   |
|    | 2001 | TTCTGGAAAT  | TCAGCAACAG  | CAACAGCAAC  | AACACTACAC  | GATCAAGAGG   |
|    | 2051 | GTGTGGTGGG  | AGCGATCTCT  | TGTAATATCT  | CAGAGCTCTGA | CATAGCTACA   |
| 45 | 2101 | AAAAGCTTAA  | CTCTTACTGA  | AAATGAGAGT  | TTAAGTTTCA  | TTAACAATAC   |
|    | 2151 | GGCAAAAAGA  | AGTGGTGGTG  | GTAATTTATGC | TCCTAAGTGT  | GTAATCTCAG   |
|    | 2201 | CGATGGAATC  | CATAAACTTT  | GATGGCAATA  | CTGCTGAATC  | TTGGGAGGGA   |
|    | 2251 | CGCATTTAAT  | GCAAAAACCT  | TTCGATTACA  | GCTAACGGTC  | CTGTCTCTCT   |
|    | 2301 | TACCAATTAAT | CTTGGAGGCA  | AGGGAGGGCG  | CAATTTATATA | GCCGATAGCG   |
|    | 2351 | GAGCACTTTT  | CTTAGAGGCT  | ATTGATGGGG  | ATATTACTCTT | CTCAGGGAAC   |
| 50 | 2401 | CGAGCGAGCTG | AGGGAACCTTC | AACTCCCAAC  | TGCAATCMTAT | TAGGTCGAGG   |
|    | 2451 | GGCTAAGATC  | ACTAAGCTTG  | CAGCAGCTCC  | TGGTCAATAG  | ATTATTTTTT   |
|    | 2501 | ATGATCCTAT  | TACGATGGAA  | GCCTCTGCAT  | CTGGAAGAAC  | AATAGAGGAG   |
|    | 2551 | TTAGTCATCA  | ATCTGTTGTG  | CAAAAGCTAT  | GTCTCTCCTC  | CCCAACCAAA   |
|    | 2601 | AAATGGTCTCT | ATAGCTTCAG  | TGCTGTGATG  | CCCTGTAGCA  | CTCCTGAACC   |
| 55 | 2651 | CAAAACCGGG  | AACTATAGTA  | TTTCTCTCTG  | GAAATCTCCC  | CRGTCAAGAT   |
|    | 2701 | GGCTCGATTC  | CTGCAAAATC  | TACCAACATA  | CTGAACAGAA  | AGATCAAACT   |
|    | 2751 | AGCAGGAGGA  | AATGTCTGTT  | TAAAGAGAGG  | AGGCCCTCTA  | CAAGTATATAT  |
|    | 2801 | CCTTCACACA  | GCAGCTCGAT  | TCTACAGAT   | TCA.TGGATGC | AGGAACGACC   |
|    | 2851 | TTAGAGACCA  | CGCAACATTA  | CAATACAGAT  | GGCAGCATCG  | ATCTAAAGAA   |
| 60 | 2901 | TCTCTCTGTA  | AATCTGGATG  | CTTATAGTGG  | CAAGCGTATG  | ATAACGATTT   |
|    | 2951 | CCGTAAACAG  | CACAAGTGGG  | GGATTAAAJA  | TCTCAGGGGA  | TCTGAJAATTC  |
|    | 3001 | CATACAATG   | AAAGGAAGTT  | CTATGACAAAT | CTTGGGTGTA  | AAGCAAACTT   |
|    | 3051 | AAATCTTCTCT | TTCTTAGATC  | TTTCTTCTAC  | TTCAGGAACCT | GTAJAATTTAG  |
|    | 3101 | ACGACTTCAA  | TCCGATTCCT  | TCTAGCATGG  | CTGCTCCGGA  | TTTGGGTATG   |
| 65 | 3151 | CAAGGGAGTT  | GGACTCTGGT  | TCTTAAAGTA  | GGAAGCTGGG  | GGAGGTGAC    |
|    | 3201 | TTTGGTCCGG  | GAATGGCAAG  | CGTTTAGGATA | CACCTCTTAA  | CCAGAGCTTC   |
|    | 3251 | GTGGCACTTT  | AGTTCCTAAT  | AGCCTTGGGA  | ATGCTTATGT  | ATACATCCAT   |

3301 TCTATACAGC AGGAGATCGC CACTGCGATG TCGGACGCTC CCTCACATCC  
 3351 AGGGAATTGG ATTGGAGGTA TTGGCAACGC CTTCATCAA GACAAGCRAA  
 3401 AGGAAATGCG AGGATTCCCT TTGATTCCCA GAGGTATAT TGTGGTGGCG  
 3451 AGCATGACCA CCCCTCAAGA ATATACCTTT GCTGTTGAT TCAGCCAACT  
 5 3501 CTTTGGCAAA TCTAAGGATT ACSTAGCTCT GGATATTAAA TCTCAAGTCT  
 3551 ATGCAAGGAT TCTCTGTGCT CAGAGCTCTT ATGTCATCT CCTGACATGC  
 3601 TCATTACGTC GCCACGTCCT CTTAAGGTC CTTCAGAGC TCCCAGGAGA  
 3651 AACTCCCTCT GTCTCCCATG GTCAAGTTTC CTATGGAAGA AACCACATA  
 10 3701 ATATGACGAC AAAGCTTTCG AAACACACAC AAGGGAATCT AGACTGGGAC  
 3751 AGCCATAGCT TCGCTGTGTA AGTCGGTGGT TCTCTCTCG TAGACTCTAA  
 3801 CTACAGATAC CTTACAGAGT ACCTGCCCCA TGTGAATCT CAAGTGTGTA  
 3851 GTGTAATACA AAAAGGATTC CAAGAGGTGG CTGTGATCC ACOTATCTTT  
 3901 GACGCTAGCC ATCTGGTCAA CGTGTCTATC CCTATGGGAC TCACCTTCAA  
 3951 ACACGAATCA GCAAAGCCCC CCAGTGCTTT GCTTCTTACT TTAGGTTAGC  
 15 4001 CTGTAGATGC TTACCGGGAT CACCCCTCACT GCCTGACCTC CTTAACAAAT  
 4051 GGCACCTCGT GGTCACGCTT TGCTACRAAC TTATCACGAC AAGCTTTCTT  
 4101 TGTCTAGGCT TCTGTGACAT TGAGCTTACT TCATGGTCTT GACTGCTTCG  
 4151 CTTCTGGAAG TTGTGAAGTC CGCAGCTCCT CAAGAAGCTA TAATGCAAA  
 4201 TGTGGAAGCT GTTATTCTTT CTAA

20 The PSORT algorithm predicts an outer membrane location (0.915).

The protein was expressed in *E. coli* and purified as a his-tag product, as shown in Figure 16A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 16B) and for FACS analysis (Figure 16C). A GST-fusion protein was also expressed.

The cp6727 protein was also identified in the 2D-PAGE experiment (Cpn0444).

25 These experiments show that cp6727 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 17

The following *C. pneumoniae* protein (P1D 4376731) was expressed <SEQ ID 33; cp6731>:

1 MXSSLHWFLI SSSLALPLSL NFSAPAAVVE INLGPTNSFS GPQTYTPPAQ  
 51 TTNADGTIYN LTGDVSIINA GSPTALTASC FRKTTGNLFS QGHGYQFLQ  
 101 NIDAGANCTP TNTAANKLLS FSGFSYLSLI QTNATTGTG AIKSTGACSI  
 151 QSNVSCYFGQ NFSNENGGAL QGSSISLSLN PNLTFAPNKA TQKGGALYST  
 201 GGTITNNLNL SASFSENTAA NNGGAIYTEA SSFISNNKAI SPINNVSVTAT  
 251 SATGGAAYCS STSAPKPVLT LSDNGLNFI GNTATTSGGA IYTDNLVLSS  
 301 GGPTLFKNNS AIDTAAPLGG AIALADSSGL SLALGGDIT FEGNTVVKGA  
 351 SSSQTTTRNS INIGNTNAKI VQLRASQNT IYFYDPTTS ITAALSDALN  
 401 LKNGPDLAGNP AYQCTIVFSG EKLSEAEAAE ADNLKSTIQ PLTLAGGQLS  
 451 LKSGVTLVAX SFSQSPGSTL LMDAGTTLET ADGTTINNLV LNVDSLKREK  
 501 KATLKATQAS QVTLSGSL S LVDPSGNVYE DSWNNNPQV SCLTLTADDP  
 551 ANIHITDLAA DPLEKNPIHW GYQGNWALSW QEDTAKSKA ATLTWTKTGY  
 601 NENPERRGTL VANTLWGSFV DVSRTQQLVA TKVRQSGQTR GIWCBGISNF  
 651 PHKDSTKINK GPRHISAGYV VGATTTLASD NLITAAFPCL FGKDRDHPIN  
 701 KNRASAYAAS LHLQHLAFLS SPSSLRLYLP SRSQPVLPD AQLSYIYSKN  
 751 TMKTYTQAP KGESSWYNDG CALELASSLP HTALSHGLF HAYPPFIKVE  
 801 ASYIHQDSPK ERNTVLVRSF DSGDLINVSF PIGITFERFS RNBRASYEAT  
 851 VIYVADVVRK NPDCTALLI NMTSWKTGTG NLSRQAGIGR AGIFYAFSPN  
 901 LKVTSLMSME IGRSSRSYNA DLGGKQFQ\*

A predicted signal peptide is highlighted.

The cp6731 nucleotide sequence <SEQ ID 34> is:

50 1 ATGAATCCT CTCTTCATTG GTTTTAAATC TCGTCATCTT TAGCACTTCC  
 51 CTCTCAGCTA AATTCTCTCG CGTTTGTCTG TGTGTTGAA ATCAACTTAG  
 101 GACCTACCAA TAGCTCTCTC GGACACAGAA CCTACACTCC TCCAGCCCAA  
 151 ACACAAATG CATAGGGAAC TATCTATAAT CTAAACAGGG ATGTCTCAAT  
 201 CACCAATGCA GGATCTCCGA CAGCTCTAAC CGCTCTCTGC TTTAAAGAAA

|      |             |             |             |             |             |
|------|-------------|-------------|-------------|-------------|-------------|
| 251  | CTACTGGGAA  | TCTTTCTTTTC | CAAGGCCACG  | GCTACCAATT  | TCTCCTACAA  |
| 301  | AATATCGATG  | CGGGAGCGAA  | CTGTACCTTT  | ACCAATACAG  | CTGCAAAATAA |
| 351  | GCTTCTCTCC  | TTTTTCAGGAT | TCCTCTATT   | GTCACTAATA  | CAAAACCAGGA |
| 401  | ATGCTACCAC  | AGGAACAGGA  | GCCATCAAGT  | CCACAGGAGC  | TTGTTCTATT  |
| 451  | CAGTCGAAC   | ATAGTTGCTA  | CTTTGGCCAA  | AACTTTTCTTA | ATGACAAATGG |
| 501  | AGGCGCCCTC  | CAAGGCAGCT  | CTATCAGTCT  | ATCGCTAAAC  | CCCAACCTTAA |
| 551  | CGTTTGGCAA  | AAACAAGACA  | ACCGAAAAAG  | GGGGTGCCCT  | CTATTCCACAG |
| 601  | GGAGGGATTA  | CAATTAAACA  | TACGTAAAC   | TCAGCATCAT  | TTTCTGAAAA  |
| 651  | TACCGCGGCG  | AACATGGCG   | GAGCCMTTAA  | CACGGAAAGT  | AGCATTTTAA  |
| 701  | TTAGCAGCAA  | CAAGCAAAAT  | AGCTTTTATA  | ACAAATAGTGT | GACCGCAACC  |
| 751  | TCAGCTACAG  | GGGGAGCCAT  | TTACTGTAGT  | AGTACATCAG  | CCCCCAAAAC  |
| 801  | AGCTTTAACT  | CTATCAGACA  | ACGGGGAAC   | GAACTTTATA  | GGAAATACAG  |
| 851  | CAATTACTAG  | TGGTGGGCGC  | ATTATACTAG  | ACAACTAGAT  | TCTTCTCTCT  |
| 901  | GGAGGACCTA  | CGCTTTTATA  | AAACAACCTCT | GCTATAGATA  | CTGCAGCTCC  |
| 951  | CTTAGGAGGA  | GCAATTGCGA  | TTGCTGACTC  | TGGAATCTTTG | AGTCTTTGCG  |
| 1001 | CTCTTGGTGG  | AGACATCACT  | TTTGAAAGGAA | ACACAATAGT  | CAAAAGGAGCT |
| 1051 | TCCTTCGAGTC | AGACCACATAC | CAGAAATTCCT | ATTAAACATCG | GAAACACCAA  |
| 1101 | TGCTTAAGATT | GTACAGCTGC  | GAGCCCTCTCA | AGGCAATATCT | ATCTCATCTCT |
| 1151 | ATGATCCTAT  | AACAACATAGC | ATCACTGCGC  | CTCTCTCAGA  | TGCTCTAAAC  |
| 1201 | TTAAATGGTC  | CTGACCTTGC  | AGGGAATCCT  | GCAATATAGAT | GAAACATCTGT |
| 1251 | ATTTTCTTGA  | GAGAGCTCTT  | CGGAAGCAGA  | AGCTGCAGAA  | GCTGATAATC  |
| 1301 | TCAAATCTAC  | AAITTCAGCAA | CCCTCTAACTC | TTGCGGGAGG  | GCAACTCTCT  |
| 1351 | CTTAATCTAG  | GAGTCACTCT  | AGTTGCTAAG  | TCTTTTTCGC  | AMTCTCCGGG  |
| 1401 | CTCTACCCCT  | CTCATGGATG  | CAGGGACCAAC | ATTAGAAACC  | CTGATGGGA   |
| 1451 | TCACATACAA  | TAATCTTGTT  | CTCAATGTAG  | ATTCTCTTAA  | AGAGACCAAG  |
| 1501 | AAGGCTACGC  | TAAAGCAAC   | ACAAGCAAGT  | CAGACAGTCA  | CTTTATCTGG  |
| 1551 | ATCGCTCTCT  | CTGTGTAGATC | CTTCTGGAAA  | TGTCACAGAA  | GATGTCTCTCT |
| 1601 | GGAAATACCC  | TCAAGTCTTT  | TCCTGTCTCA  | CTCTTACTGC  | TGACGACCCC  |
| 1651 | GCGAATATTC  | ACATCAGAGA  | CTTAGCTGCT  | GATCCCGTAG  | AAAAAAATCC  |
| 1701 | TATCCATTGG  | TGGATCCAAG  | GGAAATTTGGC | ATTATCTTGG  | CAAGAGGATA  |
| 1751 | CTGCGACTAA  | ATCCAAAGCA  | CGCACTCTTA  | CCTGGACAAA  | AACAGGATAC  |
| 1801 | AATCCGAACT  | CTGAGCGTGC  | TGGAACCTTA  | GTGTCATAAC  | CGCTATGGGG  |
| 1851 | ATCTCTTGTT  | GATGTGCGCT  | CCATACAACA  | GCTTGTAGCC  | ACTAAAGTAC  |
| 1901 | GCCAAATCTCA | AGAAACTCGC  | GGCATCTGGT  | GTGAAGGGAT  | CTCGAACCCT  |
| 1951 | TTCCATAAAG  | ATAGCACGAA  | GATATAATAA  | GGTTTCTGCC  | ACATAAGTGC  |
| 2001 | AGGTTATGTT  | GTAGGAGCGA  | CTACAACATT  | AGCTCTGAT   | AACTCTATCA  |
| 2051 | CTGCGAGCTT  | CTGCCAATTA  | TTCCGGGAAA  | ATAGAGATCA  | CTTTATAAAT  |
| 2101 | AAAAATAGAG  | CTCTTGCTTA  | TGCAGCTTCT  | CTCCATCTCC  | AGCATCTAGC  |
| 2151 | GACCTTGCTCT | TCTCCAGGCT  | TGTTAGCGTA  | CCTTCCTGGA  | TGTGAAAGTG  |
| 2201 | AGCAGCCGPT  | CCTCTTTGAT  | GCTCAGATCA  | GCTATATCTA  | TGTTAAAAAT  |
| 2251 | ACTATGAAA   | CCTATTACAC  | CCAAGCACCA  | AAGGGAGAGA  | GCTCTGTGTA  |
| 2301 | TAAATACGPT  | TGCTCTCTGC  | AACCTTGAGG  | CTCCCTACCA  | CACACTGCTT  |
| 2351 | TAGGCCAAGA  | GGGTCTCTTC  | CACGGGTATT  | TTCTCTTCAAT | CAAAGTAGAA  |
| 2401 | GCTCTGTACA  | TACACCAAGA  | TAGCTTCAAA  | GAAGCTAATA  | TACCTCTTGT  |
| 2451 | ACCACTTTTC  | GATAGCGGTG  | ATTTAAATTA  | GTCTCTGTGT  | CGTATTTGGA  |
| 2501 | TTACCTTTGGA | GAGATTCTTC  | AGAAACGAGC  | GTGCTCTCTA  | CGAAGTACT   |
| 2551 | GTCACTTAGG  | TGCGGAGTGT  | CTATCTTAAG  | AACTCTGACT  | GCACGACAGC  |
| 2601 | TCTCTTAATC  | AACATACCT   | CGTGGAAAC   | TACAGGAACG  | AACTCTTCAA  |
| 2651 | GACAAAGCTGG | TATCCGAAGA  | CGAGGAGTCT  | TTTATGCTCT  | CTCTCCAAAT  |
| 2701 | CTTGAGGTCA  | CAGGTAACT   | ATCTATGGAA  | ATTCTGGGAT  | CTTCACGACG  |
| 2751 | CTACAAATGCA | GATCTTGGAG  | GTAAGTTCCA  | GTCTTAA     |             |

The PSORT algorithm predicts an outer membrane location (0.926).

The protein was expressed in *E.coli* and purified as a his-tag product, as shown in Figure 17A. A GST-fusion protein was also expressed. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 17B; his-tag) and for FACS analysis (Figure 17C; his-tag and GST-fusion).

The GST-fusion protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis. Less cross-reactivity was seen with the his-fusion.



These experiments show that cp6731 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 18

The following *C.pneumoniae* protein (PID 4376737) was expressed <SEQ ID 35; cp6737>:

```

5      1  MFLSFKSSSF CLLACLCSAS CAFARTRLGG NFVPTITNGQ BEILLTSDFV
51     51  CSNFLGASFS SSFINSSSNL SLLGRGLSLT PTSCQAPTHS NVALLSAET
101    101 LTFKNFSSIN PTGNQSTGLG GLYKGDIIVF QSIKDLIPTT NRVAYSASV
151    151 TTSATPAITT VTGASALQPD TDSLTVENIS QSIKFFGNLA NFGSAISSSP
201    201 TAVVKFINNT ATMSFSHNFT SSGGGVIYGG SLLFENNNG CIIFTANSCV
10     251  NSLKGVTPSS GTVALGSGGA ICIPGTFFEL KNNQCKTFS YNQTNDAGA
301    301 IYAETCNIVG NQGALLLDSN TAARNNGAIC AKVLNIQGRG PIEFSRNRAE
351    351 KGGAIPIGPS VGGPAKQTST LTLASEGDI AFQGNMLNAT PGIRNAITVE
401    401 AGGEIVLSLA QGGSRVIFYD PITHSLPTTS PSNKDITINA NGASGSVVFT
451    451 SKGLSSSTELL LPANTITILL GTVKIASBGL KITDNVAVIV LGFATQGSQ
15     551  LTLGSGGTGLG LATPTGAPAA VDTTIGKLA DPFSFLKRDV VSASVNAGTK
551    551 NVTLTGLALVL DEHVDITDLY MSLQTPVAI PIAVFKGATV TKTGFPDGEI
601    601 ATPSHYVGQW KWSYTWRSPL LIPADPGGFP GGPSANTL YAVVNSDITLV
651    651 RSTYILDPER YGEIVNSLW ISFLGNQAFS DILQDVLLID HPLGISITAKA
701    701 LGAYVEHTPR QGHEGFSGRY GGYQAALSMN YTDHTTLGLS PGQLYGKTNA
20     751  NPYDSRCSEQ MYLLSFFGQF PIVTKQSEAL ISWKAAYGS KNHLNITYLR
801    801 PDKAPKSGQG WHNNSYVYLI SAEHPFLNWC LLTRPLAAGN DLGSIISAEF
851    851 LGGWQSKFTE TGDQLQSFSSR GKGYNVSLPI CGSSQWTFPF KKAPSTLATIK
901    901 LAYKPDIVRY NPHNIVTVVS NQESTSISGA NLRHHGLFVQ IHDVVDLTED
951    951 TQAFPLNYTFD GKNGFTNHRV STGLKSTF*

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25 A predicted signal peptide is highlighted.

The cp6737 nucleotide sequence <SEQ ID 36> is:

```

1      1  ATGCCCTTTT CTTTCAAACT TTAATCTTTT TGCTTACTTG CCGTGTATTG
51     51  TAGTGCAAGT TGCGCGTTTG CTGAGACTAG ACTCGAGAGG AACTTTGTTT
101    101 CTCCAAATAC GAATCAGGGT GAAGAGATCT TACTCACTTC AGATTTTGTG
151    151 TGTTCAAACT TCTTGGGGGC GAGTTTTTC AAGTCCCTTTA TCAATAGTTC
201    201 CAGCAATCTC TCCTTATATG GAAAGGGCCT TCCTCTAAAG TTAACCTCTT
251    251 GTCAAGCTCC TACAATAGT AACATATGCG TACTTCTCGC CGCAGAGACT
301    301 CTGACCTTCA AGAATTTTTT TCTATAAAC TTTACGAGGA ACCAATCGAC
351    351 AGGACTTTGG GGCCTCATCT ACGGAAAAGA TATGTGTTTC CAATCTATCA
401    401 AAGATTGAT CTCACTACAG AACCGTGTTG CCTATTCTCC AGCATCTGTA
451    451 ACTACGTCGG CAACCTCCGC AATCACTACA GTAACACAG GAGCCTCTGC
501    501 TCTCCAAACC ACAGACTCAC TCACTGTGCA AAACATATCC CAATCGATCA
551    551 AGTTTTTTGG GAACCTTGCC AACTTCGGCT CTGCAATTAG CAGTTCTCCC
601    601 ACGCGAGTCG TAAATTCAT CAATACACCC GCTACCATAG GCTTCTCCCA
40     651  TAACTTTACT TCGTCAGGAG GCGGCGTAT TTAATGAGGA AGCTCTCTCC
701    701 TTTTGTAAAA CAATCTGGA TGCAATCATCT TCACCGCCAA CTCCTGTGTG
751    751 AACAGCTTAA AAGGCGTAC CCCTTCATCA GGAACCTATG CTTTAGGAAG
801    801 TGGCGGAGCC ATCTGCATCC CTACCGGAC TTTGAAATA AAAAACAATC
45     851  AGGGGAGTGT CACCTTCTCT TATAATGTA CACCAAAATG TCGGGGTGCG
901    901 ATCTACGCGG AAACCTGCAA CATCTTAGGG AACCAAGGTT CTTTGTCTCT
951    951 AGATAGCATC ACTGACGCGA GAAATGCGCG AGCCAICTGT GCTAAAGTGC
1001   1001 TCAATATATC AGGACGCGGT CCTATTGAAT TCTCTAGAAA CCGCGGGAG
1051   1051 AAGGCGGAG CTATTTCAT AGGCCCTCTT GTTGGAGACC PTCGGAAGAG
1101   1101 AACATCGACA CTACGATTTT TGGCTTCCGA AGGTGATATT CGGTTCAGAG
50     1151  GAACATPGCT CANTACAAA CTTGGAATCC GCAATGCCAT CACTGTAGAA
1201   1201 GCGGCGGAG AGATTGTGT TCTATCTGCA CAAGGAGGCT CACTGTGTGT
1251   1251 ATTTTATGAT CCATTACAC ATAGCTCCCC AACCACAGAT CGCTCTATA
1301   1301 AAGACATTAC TCTCTCTTAC AGATCGCTTT CAGCATCTGT AGCTCTTAC
1351   1351 AATAGGGAC TCTCTCTTAC AGAATCTCTG TTGCTCTGCT ACACGACAC
55     1401  TATACTTCTA GGAACATCA AGATCGCTAG TGGACCTGCA AGATCTACTG
1451   1451 ACAATGCGGT TGTCAATGTT CTATGCGTTG GTTATGAGAA GTTAGCATTC
1501   1501 CTTACCTTGG GCTCTGAGAG ACCCTTAGGG CTTGACATG CAGTAAATGC
1551   1551 ACCTGCGGCT GTAGACTTTA CQATTTGAAA GTTAGCATTC GATCCTTTT
1601   1601 CCTTCTCTAA AAGAGATTTT GTTTCAGAT CAGTAAATGC AGSCACAAA
60     1651  AACCTCATT TAAACGAGAG TCTGTTCTTT GATGACATG ACGTTACAGA

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|      |            |            |            |             |             |
|------|------------|------------|------------|-------------|-------------|
| 1701 | TCTTTATGAT | ATGGTGTCAT | TACAAACTCC | AGTAGCAATT  | CCTATCGCTG  |
| 1751 | TTTTCRAAGG | AGCAACCGTT | ACTAAGACAG | GATTTCCTGA  | TGGGGAGATT  |
| 1801 | CGCATCCCAA | GCCCATACGG | CTACCAAGGA | AAGTGGTCCT  | ACACATGGCT  |
| 1851 | CCGTCGCCCT | TTAATTCAG  | CTCCTGATGG | AGGATTTTCCT | GGAGGTCCCT  |
| 1901 | CTCCTAGCGC | AAATACTCTC | TATGCTGTAT | GGAAATTCAGA | CACCTCTCGG  |
| 1951 | CGTTCTACCT | ATATCTTAGA | TCCCAGCGT  | TACGGAGAAA  | TTGTTCAGCAA |
| 2001 | CAGCTTATGG | ATTTCCTTCT | TAGGAATATC | GGCATTTCTCT | GATATTTCCT  |
| 2051 | AAGATGTCTT | TTTGATAGAT | CATCCGGGT  | TGTCATATAC  | CGCGAAAGCT  |
| 2101 | TTAGGAGCCT | ATGTCGAACA | CACACCAAGA | CAAGGCATG   | AGGGCTTTTC  |
| 2151 | AGGTGCTGAT | GGAGGCTACC | AAGCTGCGCT | ATCTATGAAC  | TACACGGACC  |
| 2201 | ACACTACGTT | AGGACTTTCT | TTCCGGGAGC | TTTATGGAAA  | AACTAACGCC  |
| 2251 | AACCCCTACG | ATTCACTGTC | CTCAGAACAA | ATGTATTTAC  | TCTCGTTCCT  |
| 2301 | TGGTCAATTC | CCTATGCTGA | CTCAAAGAG  | CGAGGCCTTA  | ATTTCCTGGA  |
| 2351 | AAGCAGCTTA | TGGTTATTC  | AAAAATCACC | TAAATACCAC  | CTACCTCAGA  |
| 2401 | CCTGACAAAG | CTCCAAATC  | TCAAGGCAAA | TGGCATATACA | ATAGTTACTA  |
| 2451 | TGTTCTTATT | TCTGCGAATC | ATCCTTTCT  | AACTGGTGT   | CTTCTTACAA  |
| 2501 | GACCTCTGGC | TCAAGCTTGG | GATCTTTAC  | GTTTATTTTC  | CGCAGAATTC  |
| 2551 | CTAGTGTGGT | GGCAAGATGA | GTTACAGAAA | ACTGGAGATC  | TGCAACGTAG  |
| 2601 | CTTTAGTAGA | GTTAAAGGTT | ACAATGTTTC | CCTACCGATA  | GGATGPTCTT  |
| 2651 | CTCAATGGTT | CACACATTT  | AAGAAGGCTC | CTTCTACACT  | GACCATCAAA  |
| 2701 | CTTGCTTACA | AGCCTGATAT | CTATCGTGT  | AACCCCTACA  | ATATTGTGAC  |
| 2751 | TGTCGPTCTA | AACCAAGAGA | GCATCTGAT  | CTCAGGAGCA  | AATCTACGCC  |
| 2801 | GCCACGGTTC | GTTTGTAACA | ATCCATGATG | TAGTAGATCT  | CACCGAGGAC  |
| 2851 | ACTCAGGCTT | TTCTAAACTA | TACCTTTGAT | GGGAAAAATG  | GATTTACAAA  |
| 2901 | CCACGAGTGG | TCTACAGGAC | TAAATCCAC  | ATTTTAA     |             |

The PSORT algorithm predicts an outer membrane location (0.940).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 18A. The recombinant protein was used to immunise mice, whose sera were used in an immunoblot analysis blot (Figure 18B) and for FACS analysis (Figure 18C). A his-tagged protein was also expressed.

The cp6737 protein was also identified in the 2D-PAGE experiment (Cpn0454) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6737 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 19

The following *C. pneumoniae* protein (PID 4377090) was expressed <SEQ ID 37; cp7090>:

|     |                             |             |            |            |
|-----|-----------------------------|-------------|------------|------------|
| 1   | <b>MNIHSLWKLC FLALLALPA</b> | CSLSPNYWE   | DSNCNTHHR  | RKPKSFGFV  |
| 51  | PLYTFEDFN                   | NFTTGYEYDSK | ERKQYKSSQV | AAFRNITPAT |
| 101 | LAILTMLVHY                  | MKRNPKATLY  | TEGHTDERGA | ASYNLALGAR |
| 151 | RQGISADRLS                  | TIYSYGEHPL  | NSGHNELAWQ | QNRRTFEKIH |
|     |                             |             |            | AR*        |

A predicted signal peptide is highlighted.

The cp7090 nucleotide sequence <SEQ ID 38> is:

|     |            |            |            |            |            |
|-----|------------|------------|------------|------------|------------|
| 1   | ATGAATATAC | ATTCCCTATG | GAACCTTTGT | ACTTTATTTG | CTTTACTTGC |
| 51  | ATTGCCAGCA | TGTAGCTCTT | CCCTTAATTA | TGGCTGGGAG | GATTTCTGTA |
| 101 | ATACATGCCA | TCATACAAAG | CGAAAAAAGC | CTTCTCTTPT | TGGCTTTGTT |
| 151 | CCTCTCTATA | CCGAAGAGGA | CTTTAAACCT | AATTTTACCT | TCGCTGAGTA |
| 201 | TGATTTCCAA | GAAGAAAAAC | ANTACAAGTC | AAGCCAAATT | GCACATTTTC |
| 251 | GTAATATCAC | CTTTGCTACA | GACAGCTATA | CAATTTAAGG | TGAAGAGAAC |
| 301 | CTTGCGATTC | TACGACACTT | GOTTCACTAC | ATGAAGAAAA | ACCCGAAGAC |
| 351 | TACACTGTAC | ATTGAAGGGC | ATACTGACGA | CGCTGGAGCT | GCATCTTATA |
| 401 | ACCTTGCTTT | AGGAGCACGA | CGAGCCAAAT | CGATTTAAGA | GCATCTCCGA |
| 451 | AAGCAGGGAA | TCTCTGACGA | TGCTCTATCT | ACTATTTCCT | ACGGAAGAGA |

501 ACATCCCTTTA AATTCGGGAC ACAACGAAC AGCATGGCAA CAAAATCGCC  
551 GTACAGAGTT TAAGATTCAT GCACGCTAA

The PSORT algorithm predicts an outer membrane location (0.790).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 19A.

- 5 A his-tagged protein was also expressed. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 19B) and for FACS analysis.

These experiments show that cp7090 is useful immunogen. These properties are not evident from the sequence alone.

### Example 20

- 10 The following *C. pneumoniae* protein (PID 4377091) was expressed <SEQ ID 39; cp7091>:

1 **MLROLCPQVF FFCFASLVYA** EEEVVVRSE HITLPIEVSC QTDTPDKPKIO  
51 KYLSLSTEIF KCDIALGDCL QPTAAKESSE SPLAISLRHL VPQLSVVLLQ  
101 SSKTPTOLCS FTISQNLSDV RQKIHHAADT VHYALTGIPG ISAGKIVFAL  
151 SSLGKQDKLK QGRLWTTDYG GKNLAPLTTE CSLSITPKWV GVGSNFFPLYI  
201 VSYKYGVPKI FLGSELENTG KXVLPKGNQ LMPTSPRKK LLAFLVADTVG  
251 NPDLFIQPPS LITSGPMGRPR RLLNENFTQ GNPSFNPEGQ QLVFISNKGK  
301 RPRLYIMSLD PEPQAPRLLT KKYRNSCPA WSPDGKIAF CSVIKGVROI  
351 CIYDLSSGED YQLTTSPTNK ESPSWAIDSR HLVSFGNAIE ESELYLISLV  
401 TKKTNKIAIG VGERKRFPSWG AFPQPIKRT L\*

- 20 A predicted signal peptide is highlighted.

The cp7091 nucleotide sequence <SEQ ID 40> is:

1 ATGTTACGGC AACTATGCTT CCAAGTTTTT TTCTTTTGCT TCGATCGCT  
51 AGTCTATGCT GAAGAATTAG AAGTTGTGT CGSTTCOGAA CATATCACGC  
101 TCCCTATGTA GGTCTCTTGC CAGACCGATA CGAAAGATCC AAAAATACAG  
251 AAATACCTCA GCTCGCTAAC GGAGATATTI TGCAAGGACA TTGCCCTAGG  
201 AGATTGCTCA CAACCCACAG CGGCTTCTAA AGAATCGTCA TCTCCTTAG  
251 CAATATCTTT ACGGTTGCAT GTACCTCAGC TATCTGTAGT GCTTTTACAG  
301 TCTTCAAAAA CTCTCCAAAC CTTAATGTCT TTACTATT TTCAAAATCT  
351 TTCTGTAGAT CGTCAAAAAA TCCATCACGC TGCTGATACA GTTCATTACG  
401 CCCTCACAGG GATTCTCTGA ATCAGTGCTG GGAATAATGT TTTTGTCTTA  
451 AGTTCTTTAG GAAAGATACA AAAGCTCAAG CAAGGAGAT TATGAGTAC  
501 AGATTACGAT GGAAGAAACC TCGCCCTTTT AACCACAGAA TGTCGCTCT  
551 CTATACTCC AAAATGGGTG GGTGTGGGAT CAAATTTTCC CTATCTCTAT  
601 GTTTCGTATA AGTATGGTGT GCCTAAAAAT TTCTTGTGTT CCCTGAGAAA  
651 CACTGAAGGT AAAAAGTCC TTCCGTTAAG AGGCAACCAA CTCATGCCTA  
701 CGTTTCTTCC AAGAAAAAAG CTTTGTAGCT TGTGTGCTGA TACGTATGGA  
751 AATCCTGATT TATTATTATCA ACCGTTCCTA CTAACCTCAG GACCTATGGG  
801 TCGCCACGCT CGCTCCTCTA ATGAGAAATT CGGAGACTCA GGAATCCCT  
851 CCTTCAACCC TGAAGATCC CAGCTTGTCT TTATATGAAA CAAAGACGSC  
901 CGTCCGGGTC TTTATATAT TGTCCCTCGAT CCTGAAACCC AAGCACCTCG  
951 CTTGTGACCA AAAAATACCA GAAATAGCAG TTGCCCTGCA TGGTCTCCAG  
1001 ATGGTAAAAA AATAGCCTTC TGCTCTGTAA TTAAGGGGTG GCGACAAATT  
1051 TGTATTACG ATCTCTCTCC TGGAGAGGAT TACCACTCA CTACGCTCC  
1101 CACAATTAAC GAGAGTCTCT CTTGGGCTAT AGACAGCCGT CATCTGTCT  
1151 TTAGTGGGGG GAATCTGAAA GAATCAGAGT TATATTTAAT CAGCTAGTCT  
1201 ACCAAAAAAA CTAACAAAT TGCTATAGGA GTAGGAGAAA AACGGTTCCC  
1251 CTCTGGGGT GCTTCTCCCT AGCAACCGAT AAGAGAGACA CATATGA

The PSORT algorithm predicts an inner membrane location (0.109).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 20A.

- 50 A his-tagged protein was also expressed. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 20B) and for FACS analysis.

These experiments show that cp7091 is a useful immunogen. These properties are not evident from the sequence alone.

### Example 21

The following *C.pneumoniae* protein (PID 4376260) was expressed <SEQ ID 41; cp6260>:

|    |     |                                     |             |             |            |
|----|-----|-------------------------------------|-------------|-------------|------------|
| 5  | 1   | <b>MRFSLCGPPL VPSFTLLSVF DTSLSA</b> | <b>TTIS</b> | LTPEDSFHGD  | SQNAERSYNV |
|    | 51  | QAGDVYSLTG                          | DVSIENVSDNS | ALNKACPNVT  | SGSVTFAGNH |
|    | 101 | GTTKEGAVLC                          | QDPQNTARF   | SGFSTLSFIQ  | SPGDIKEGQC |
|    | 151 | NNYVVRFEQN                          | QSKRTGGAIS  | GANVTIVGNY  | DSVSFYQNA  |
|    | 201 | PLQIAVNQAE                          | IRPAQNTAKN  | SGGGALYSDG  | DDIDQNAVY  |
| 10 | 251 | ATGKGGAYCC                          | LPTSGSSTPV  | PIVTFSDNKG  | LVFERNHISM |
|    | 301 | SISGGGPTLF                          | NNISYANSQ   | NLGGALIDT   | GGISLSAEK  |
|    | 351 | SLPFLINGIHL                         | LQNAKFLKLQ  | ARMGVSIIEFY | DPITSEADGS |
|    | 401 | NKEYTGTILF                          | SGEKSLLANDP | RDPSKTIQPN  | VNLSAGYLV  |
|    | 451 | PTQSPGSHLV                          | LDLGTKLIAS  | KEDIAITGLA  | IDISLSSSS  |
| 15 | 501 | NKQISVTDSE                          | ELISPTGNAY  | EDLRMRNSQT  | FPLLSLEPA  |
|    | 551 | FLPVSPHYGF                          | QGNWKLAWTG  | TGNKVGEFSP  | DKINYPKPRP |
|    | 601 | WGNADVVRSL                          | MQVQETHASS  | LQTDRLGLWD  | GIGNFFHVSA |
|    | 651 | SGGVVLSVNN                          | EITPKHYTSM  | AFSOLFSLRK  | DYAVSNNEHY |
|    | 701 | TTSLGNIFRY                          | ASRNPVNVMG  | ILSRFLQNP   | LMIPHFLCAV |
| 20 | 751 | YANFPMVKNS                          | WRNMCWAIEC  | GGSMFLVFE   | NGRLFCQAI  |
|    | 801 | QGDFKETTAD                          | GRRFSNGSLT  | SISVFLGIRF  | EKLALSQDVL |
|    | 851 | IFRKDPSCEA                          | ALVISGDSML  | VPAAHVSRHA  | FVSGSGTRYH |
|    | 901 | GSIECRPHAR                          | NYNINCGSKF  | RF*         | FNDTFTLLCR |

A predicted signal peptide is highlighted.

25 The cp6260 nucleotide sequence <SEQ ID 42> is:

|    |      |             |            |             |             |             |
|----|------|-------------|------------|-------------|-------------|-------------|
|    | 1    | ATGCGATTTT  | CGCTCTCGGG | ATTTCCTCTA  | GTTTTTCTCT  | TTACATGTCT  |
|    | 51   | CTCAGTCTTC  | GACACTCTCT | TGAGTGTCTAC | TACGATTCTCT | TTAACCOCAG  |
|    | 101  | AAGATAGTTT  | TCATGGAGAT | AGTCAGAAATG | CAGAACGTTC  | TTATAATGTT  |
|    | 151  | CAAGCTGGGG  | ATGTCATATG | CCTTACTGCT  | GATGTCTCAA  | TATCTAACGT  |
| 30 | 201  | CGATTAACCT  | GCATTAAATA | AAGCCTGCTT  | CAATGTGCACC | TCAGGAAGTG  |
|    | 251  | TGACGTTCGC  | AGGAATCAT  | CATGGGTTAT  | ATTTTAAATA  | TATTTCTCTCA |
|    | 301  | GGAACTACAA  | AGGAAGGGGC | TGTACTTTGT  | TGCCAAGMTC  | CTCAAGCAAC  |
|    | 351  | GGCAGCTTTT  | TCTGGGTTCT | CCACGCTCTC  | TTTTTATTCAG | AGCCCCGGAG  |
|    | 401  | ATATTAAAGA  | ACAGGGATGT | CTCTATTCAA  | AAAATGCAC   | TATGCTCTTA  |
| 35 | 451  | AACAATATAT  | TAGTGCCTTT | TGAACAAAAC  | CAAAATGAAG  | CTAAAGCGGC  |
|    | 501  | AGCTATTAGT  | GGGGCGAATG | TTACTATAGT  | AGGCAACTAC  | GATTCCGTCT  |
|    | 551  | CTTCTATCA   | GAATGCAGCC | ACTTTTGGAG  | GTGCTATCCA  | TTCTTCAGGT  |
|    | 601  | CCCCACAGAA  | TGTCAGTAAA | TCAGGCAGAG  | ATAAGATTGG  | CACAAAATAC  |
| 40 | 651  | TGCCAAGAA   | GGTCTGAGAG | GGGCTTTGTA  | CTCCGATGCT  | GATATTGATA  |
|    | 701  | TGATCAGAA   | TGCTTATGTT | CTATTTCGAG  | AAAATGAGGC  | ATTGACTACT  |
|    | 751  | GCTATTAGTA  | AGGGAGGGGC | TGCTCTGTTG  | TTTCCCACCT  | CAGGAAGTAG  |
|    | 801  | TACTCCAGTT  | CCTATTGTGA | CTTCTCTGGA  | CAATAAACAG  | TTAGTCTTTG  |
|    | 851  | AAAGAAACCA  | TTCCATAATG | GGTGGCGGAG  | CCATTATAGC  | TAGGAACACT  |
|    | 901  | AGCATCTCTT  | CAGGAGGTCC | TACTCTATTT  | ATCAATAATA  | TATCATATGC  |
| 45 | 951  | AAATTCGCAA  | AATTTAGGTG | GAGCTATTGC  | CATTGATCAT  | GAGGGGGAGA  |
|    | 1001 | TCAGTTTATC  | AGCAGAGAAA | GGAACAATTA  | CATTCCAGAG  | AAACCGAGCG  |
|    | 1051 | AGCTTACCGT  | TTTTGAATGG | CATCCATCTT  | TTACAAAATG  | CTAAATTCCT  |
|    | 1101 | GAAATACAG   | CGAGAGAAAT | GATACCTAT   | AGAAATTTAT  | GATCCTATTA  |
|    | 1151 | CTTCTGAGAC  | AGATGGGTCT | ACCCTAATGA  | ATATCAACCG  | AGATCCTAAA  |
| 50 | 1201 | AATAAAGAGT  | ACACAGGACC | CATACCTTTT  | TCGGGAGAAA  | AGAGCTACAG  |
|    | 1251 | AAACGATCCT  | AGGGATTTTA | AAATCAACAT  | CCCTCAGAAC  | GTCAACCTGT  |
|    | 1301 | CTGCAGGATA  | CTTAGTTATT | AAAGAGGGGG  | CCGAAGTCCAC | AGTTTCAAAA  |
|    | 1351 | TTACAGCAGT  | CTCCAGGATC | GCATTTAGTT  | TTAGATTTAG  | GAAACAAACT  |
|    | 1401 | GATAGCCCTT  | AAGGAAGACA | TTGCCATCAC  | AGGCCCTCGG  | ATAGATATAG  |
| 55 | 1451 | ATAGCTTAA   | CTACCTCTCA | ACAGCAGCTG  | TTATTAAAGC  | AAACACCGCA  |
|    | 1501 | AATAAACAGA  | TATCCCTGAC | GGACTCTATA  | GAACTTATCT  | CGCCTACTGG  |
|    | 1551 | CAATGCCCTAT | GAAGATCTCA | GAATGAGAAA  | TTACAGACAG  | TTCCCTCTGC  |
|    | 1601 | TCTCTTTAGA  | CGCTGAGACC | GGGGGTAGTG  | TGACTGTAA   | TGCTGGAGAT  |
|    | 1651 | TTCTTACCGG  | TAAATGCCCA | TTATGCTTTT  | CAGGCAAT    | GAAATAATAG  |
| 60 | 1701 | TTGGACAGGA  | ACTGGAAACA | AAATGGAGGA  | ATTTCTCTGG  | GTAAATAATA  |

|      |             |             |            |             |             |
|------|-------------|-------------|------------|-------------|-------------|
| 1751 | ATPATAAGCC  | TAGACCTGAA  | AAAGAAGGAA | ATTTAGITCC  | TAATATCTTG  |
| 1801 | TGGGGGAATG  | CTGTAGATGT  | CAGTCCCTTA | ATCGAGGTTT  | AAGAGACCCA  |
| 1851 | TGCATCGAGC  | TTACAGACAG  | ATCGAGGCTC | GTGGATCGAT  | GGATTTGGGA  |
| 1901 | ATTTCTTCCA  | TGTATCTGCC  | TCGGAAGACA | ATATAAGTA   | CCGTATAC    |
| 1951 | AGCGGTGGAT  | ATGTCTTATC  | TGTAAATAT  | GAGATCACAC  | CTAAGCACTA  |
| 2001 | TACTTCGATG  | GCAITTTCCC  | ACTCTTTAG  | TAGAGACAG   | GACTATGCGG  |
| 2051 | TTTCCACAA   | CGAATACAGA  | ATGATTTAG  | GATCGTATCT  | CTATCAATAT  |
| 2101 | ACAACCTCCC  | TAGGGAATAT  | TTTCCGTTAT | GCTTCGCGTA  | ACCTTAATGT  |
| 2151 | AAACGTGGGG  | ATCTCTCCAA  | GAAGGTTCCT | TCAAAATCCCT | CTTATGATTT  |
| 2201 | TTCAITTTTT  | GTGTGCTTAT  | GCTCATGCCA | CCAATGATAT  | GAAACACAG   |
| 2251 | TACGCAAAIT  | TCCCTATGGT  | GAAAGACAGC | TGGGAAGACA  | ATTTGTTGGG  |
| 2301 | TATAGAGTGG  | TGAGGGGAGCA | TGCTCTTAT  | GGTATTTGAG  | AACGGAAGAC  |
| 2351 | TTTTCCAAAG  | TGCCATCCCA  | TTTATGAAC  | TACAATTAGT  | TTATGCTTAT  |
| 2401 | CAGGGAGATT  | TCAAAGAGAC  | GACTGCGAT  | GGCCGTAGAT  | TTAGTAAATGG |
| 2451 | GAGTTTAAAC  | TCAATTTCTG  | TACTCTTAG  | CATACGCTTT  | GAGAAGCTGG  |
| 2501 | CACITTTCTCA | GGATGTACTC  | TATGACTTTA | GTTTCTCCTA  | TATTCCTGAT  |
| 2551 | ATTTTCCGTA  | AGGATCCCTC  | ATGTGAAGCT | GCTCTGGTGA  | TTAGCGGAGA  |
| 2601 | CTCTGGCTTT  | GTTCGGGAC   | CACAGTATC  | AAGACATGCT  | TTTGTAGGGA  |
| 2651 | GTGGAACGGG  | TCGGTATCAC  | TTTAACGACT | ATACGTAGCT  | CTTATGTGGA  |
| 2701 | GGAAGTATAG  | AATGCCGCC   | CCAATGCTAG | AATTATATA   | TAACTGTGG   |
| 2751 | AAGCAATTT   | CCTTTTATG   |            |             |             |

The PSORT algorithm predicts an outer membrane location (0.921).

The protein was expressed in *E. coli* and purified both as a his-tag and GST-fusion product. The GST-fusion is shown in Figure 21A. This recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 21B) and for FACS analysis (Figure 21C).

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6260 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 22

The following *C. pneumoniae* protein (PID 4376456) was expressed <SEQ ID 43; cp6456>:

|     |            |             |            |             |            |
|-----|------------|-------------|------------|-------------|------------|
| 1   | MSSPVNNTPS | APNIPAPPT   | TPGIPTTKPR | SSPIEKVII   | AXYILPAIAA |
| 51  | TSAGALGILG | LSGALTPGIG  | IALLVIFVVS | MVLLGLIKD   | SISGGERLLK |
| 101 | REBVSRTFSE | NQLTVITFT   | LETEVKDLKA | AKDQLTELE   | AFRNGENNLK |
| 151 | TTADLEBEQV | SKLSEQLLEAL | ERINQLIQAN | AGDAQEISSE  | LKKLISGWDN |
| 201 | KVVEQINTSI | QALKVLGLQE  | WVQEAQTHVK | AMQEQIALQ   | AEILGHMNGS |
| 251 | TALQKSVENL | LVQDQALFRV  | VGELLESENK | LSQACSAALRQ | ETEKLAQHET |
| 301 | SLQQRIDAML | AQBQNLAEQV  | TALEKMKQBA | QKAESFEIAC  | VRDRTPGRRE |
| 351 | TPFPPTPVVE | GDESQREDEG  | GTFPVSPSS  | PVDRATGDOG  | *          |

The cp6456 nucleotide sequence <SEQ ID 44> is:

|     |             |             |            |             |             |
|-----|-------------|-------------|------------|-------------|-------------|
| 1   | ATGTCACTCTC | CTGTAAATAA  | CACACCTCA  | GCACCAACAA  | TTCCATATACC |
| 51  | AGCGCCACAG  | ACTCCAGGTA  | TTCCATACAA | AAACCTCGTT  | TCTAGTTTCA  |
| 101 | TTGAAAGAGT  | TATCATGTGA  | GCTAAGTACA | TACTATTTGC  | AATTCAGACC  |
| 151 | ACATCAGGAG  | CATCTCGAAC  | AMTCTTAGTT | CTATCTGGAG  | CGCTAACCCC  |
| 201 | AGGAATAGGT  | ATTGCCCTTC  | TTGTATCTTT | CTTTGTTTCTT | MTGGTCTTTT  |
| 251 | TAGGTTTAAAT | CCTTAAAGAT  | TCTATAGATG | GAGGAGAGAGA | ACGCAAGGCTC |
| 301 | AGAGAAGAGG  | TCTCTCGATT  | TACAAGTGAG | AACTCAACGGT | TGACAGTCAT  |
| 351 | AACCAACAA   | CTTGAGACTG  | AAGTAAAGGA | TTTAAAGCA   | GCTAAGATTC  |
| 401 | AACTTACACT  | TGAAATCGAA  | GCATTTAGAA | ATGAAAACGG  | TAAATTAATA  |
| 451 | ACAACCTGCTG | AGGACCTTAGA | AGACGAGGTT | TCTAACTTGA  | CGCAACAATT  |
| 501 | AGAAGCACTA  | GAGCGAATTA  | ATCAACTTAT | CCAAGCAAAAC | GCTGGAGATG  |
| 551 | CTCAAGAAAT  | TTCTGCTGAA  | CTAAAGAAAT | TAAATAGCGG  | TTGGGATTTCC |
| 601 | AAAGTTGTTG  | AACAGATAAA  | TACTTCTPAT | TAAAGCAATGA | AAGTGTATTAT |
| 651 | GGGTCAAGAG  | TGGGTGCAAG  | AGGCTCAAAC | ACACGTTTAA  | GCAATGCAAG  |
| 701 | AGCAAAATCA  | AGCATTGCAA  | GCTGAAATTC | TAGGAATGCA  | CAATCAATCT  |

751 ACAGCATTGC AAAAGTCAGT TGAGAATCTA TTAGTACAAG ATCAAGCTCT  
 801 AACACAGAGTA GTAGGTGAGT TGTTAGAGTC TGAGAACAAG CTAAGCCAAAG  
 851 CTGTGTTCTGC GCTACGTCAC GAAATAGAAA AGTTGGCCCA ACATGAACAA  
 901 TCTTTGCAAC AACGTATTGA TCGATGCTA GCCAAGAGC AAAATTGGC  
 951 AGAGCAGGTC ACAGCCCTTG AAAAANTGAA ACAGAAGCTC CAGAAGCCTG  
 1001 ACTCCGAGTT CATCTGCTTG GTACGTGATC GAACCTTCGG ACCTCGTGAA  
 1051 ACACCTCCAC CAACACACC TGTATGTGAA GGTGATGAAA GTCAAGAAGA  
 1101 AGACGAAGGA GGTACTCCCC CAGTATCACA ACCATCTTCA CCCGTAGATA  
 1151 GAGCAACAGG AGATGGTCAG TAA

10 The PSORT algorithm predicts inner membrane (0.127).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 22A.

The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 22B) and for FACS analysis (Figure 22C). A his-tag protein was also expressed.

These experiments show that cp6456 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 23

The following *C.pneumoniae* protein (PID 4376729) was expressed <SEQ ID 45; cp6729>:

1 MKIPLHLII SSTLVTPILL SIATYGADAS LSPTDSFDGA GGSTTFPKST  
 51 ADANGTNYVL SGNVYINDAG KGTALTGCCF TETTGDLTFT KKGYSFSFNT  
 101 VDAGSNAGAA ASTTADKALT FTGFSNLSFI AAPGTTVASG KSTLSSAGAL  
 151 NLTDNGTILF SQNVSNSEANN NGGALTTKTL SISGNTSSIT FTNSAKKILG  
 201 GAIYSSAAAS ISGNTGQIVF MNNKGETGGG ALGFEASSI TQNSLFFSG  
 251 NTAEDAAGKG GAIYCEKTE TPTLTISGNK SLTFAENSSV TQGGICAHG  
 301 LULSAAAGPTL FSNNRCGWTA AGKGGATAIA DSGSLSLSAN QGDIITPLNT  
 351 LSTSAAPTST RNAIYLGSSA KITNLRAAGQ QSIYFYDPIA SNTTASADV  
 401 TINQPDNSNP LDYSGTIVFS GEKLISADEAK AADNFTSILK QPLALASGTL  
 451 ALKGNVELDV NGPTQTGSGT LLMQPGTKLK ADTEAISLTK LVVDLSALEG  
 501 NKSYSIETAG ANKTIPLTSP LVFQDSSGNF YESHTINQAF TQPLVVFTEA  
 551 TAAADYIDA LSTSPVQTFE PHYGQGHWE ATWADTSTAK SGTMVWVTG  
 601 YNPNPERRAS VVPDSLWASF TDIRTLQQIM TSQANSIYQQ RGLNASGTAN  
 651 FPHKDKSGTN QAFRHKSYGV TVGGSADPFS ENIFSVAFQF LFGKDKDLFI  
 701 VENTSHNYLA SLYLQHRAFL GGLPMPSPFGS ITTMLKDIPL ILNAQLSYSY  
 751 TKNDMDTRYT SYPEAQGSWT NNSGALLEGG SLALYLPKEA PFFQGYFPFL  
 801 KFAQVYSRQG NFKESGAEAR AFDDGDIIVNC SIPVGIPLRK ISEDEKNPFE  
 851 ISLAYIGDVY RENPRSRTSL MVSGASWTSL CKMLARQAPL ASAGSHLTL  
 901 PHVLSGEAA YELRGSARTY NVDCOLRYSF \*

A predicted signal peptide is highlighted.

The cp6729 nucleotide sequence <SEQ ID 46> is:

1 ATGAAAATAC CCTTGCACAA ACTCTGATC TCTTGACTAC TTGCTACTCC  
 51 CATTCATTAT AGCATTTGCAA CTTACGAGAC AGATGCTCTCT TTATCCCCCTA  
 101 CAGATAGCTT TGATGGAGCG GCGCGCTCTA CATTTACTCC AAAATCTACA  
 151 CGAGTSGCCA ATGGAAACGAA CTATGTCTTA TCAGGAAATG TCTATATAAA  
 201 CGATGCTGGG AAGGCGACAG CATTAACAGG CTGCTGCTTT ACAGAAACTA  
 251 CGGTGTATCT GACATTTACT GGAAGGGAT ACTCATTTTC ATXACAACAG  
 301 GTAGATGCGG GTTCGAATGC AGAGAGCTGG GCAAGACAAA CTGCTGATAA  
 351 AGCCCTAACCA TTCACAGGAT TTCTTAACCT TTCTCTCATT GCAGCTCTCT  
 401 GAACACAGAT TGCTTCAGGA AAAAGTACTT TAAGTCTGAC AGAGGCTTAA  
 451 AATCTTACCG ATAAATGGAC GATTCCTCTT AGCCAAAAGC TCTCAATGTA  
 501 AGCTAATAAC AATGGCGGAG CGATCACCAC AAAAAGCTCT TCTATTTCTG  
 551 GGAATACCTC TCTCTATAAC TTCACTAGTA ATAGCGCAAA AAAATTAGGT  
 601 GGAGCGATCT ATGATCTCTG GCGCTGCAAGT ATTTCCAGGA ACACGGCCCA  
 651 GTTAGTCTTT ATGATTAATA AAGGAGAAAC TGGGGGTGGG GCTCTGGGCT  
 701 TTGAAGCCAG CTCCTCGAAT ACTCAAAATA GCTCCCTTTT CTTCTCTGGA  
 751 AACACTGCAA CAGATGCTGC AGGCAAGGCG GGGGCCATTT ATTCGTAAAA  
 801 AACAGGAGAG ACTCTACTCT TTACTATCTC TGGAAATAAA AGTCTGACTC  
 851 TCGCCGAGAA CTCTTCAGTA ACTCAAGGCG GAGCAATCTG TGCCCATGGT

901 CTAGATCTTT CGCGTGTGG CCCTACCCTA TTTTCAAATA ATAGATGCGG  
 951 GAAACACAGCT GCAGGCAAGG GCGGCGCTAT TGCAGTTGCC GACTCTGGAT  
 1001 CTTTAAAGTCT CTCTCGAAAT CAAGGAGACA TCACGTTCCT TGGCAACACT  
 1051 CTAACCTCAA CTTCCGCGCC AACATCGACA CGGAATGCTA TCTACCTGGG  
 1101 ATCCCTCAGCA AAAATATACGA ACTTAAGGGC AGCCCAAGCG CAATCTATCT  
 1151 ATTCTATATGA TCCGATTGCA TCTTAACCCA CAGGAGCTTC AGACGTTCTG  
 1201 ACCATTCAACC AACCGGATAG CACCTCGCCT TTAGATTTAT CAGGAACGAT  
 1251 TGTATTTTCT GGGGAAAAGC TCTCTGCAGA TGAAGCGRAA GCTGTGATA  
 1301 ACTTCACATC TATATATAAG CAACCATTTG CTCTAGCCCT TGAACCTTGA  
 1351 GCACTCAAAG GAAATGTGCA GTTAGATGTC AATGGTTTCA CACAGACTGA  
 1401 AGGCTCTACA CTCTCATATG AACCGAAGAC AAGCTCAAAA GCAGATACTG  
 1451 AAGCTATCAG TCTTACCAAA CTTGTCTGTG ATCTTCTGCG CTTAGAGGGA  
 1501 AATAAGAGTG TGTCCATTTGA AACACAGGGA GCCAACAAAA CTATAACTCT  
 1551 AACCTCTCCT CTTGTTTTCC AAGATAGTAG CGGCAATTTT TATGAAAAGC  
 1601 ATACGATAAA CCAAGCCTTC ACGCAGCCTT TGGTGGTATT CACTGCTGCT  
 1651 ACTGCTGCTA GCGATATTTA TATCGATGCG CTCTCACTTT CTCGAGTACA  
 1701 AACTCCAGAA CCTCATATCG GGTATCAGGG ACATTGGGAA GCCACTTGGG  
 1751 CAGACACATC AACTGCAAAA TCAGGAACCTA TGACTTGGGT AACTCAGGGC  
 1801 TACAACCCCTA ATCTTGAGCG TAGAGCTTCC GTAGTTCCCG ATTCAATTATG  
 1851 GGCATCTCTT ATCTGACATTG GCACCTTACA GCAGATCATG ACATCTCAAG  
 1901 CGATAGTAT CTATCAGCAA CAGGAGACTCT GGCATCAGG AACTGCGAAT  
 1951 TTTCTCCATA AGGATAAATC AGGAACTAAT CAGCATCTCC GACATAAAG  
 2001 CTACGGCTAT ATGTTTGGAG GAAGTGTCTA AGATTTTTCT GAAAATATCT  
 2051 TCAGTGTAGC TTTCTGCGAG CTCTTCGGTA AAGATAAAGA CCTGTTTATA  
 2101 GTTGAAAATA CCTCTCATAA CTATTTAGCG TCGCTATAGC TGCAACATCG  
 2151 AGCATTTCTA GGAGGACATC CCATGCCCTC ATTTTGAAGT ATCACCGACA  
 2201 TGCTGAAAGA TATTCCCTCT ATTTTGAATG CCGAGCTAAG CTACAGCTAC  
 2251 ACTAAAAATG ATATGGATAC TCGCTATACT TCCTATCTTG AAGCTCAAGG  
 2301 CTCTTGGAAC AATAACTCTG GGGCTCTAGA GCTCGGAGGA TCTCTGGCTC  
 2351 TATATCTCCC TAAAGAAGCA CCGTTCTTCC AGGGATATTT CCGCTTCTTA  
 2401 AAGTCCAGG CAGTCTACAG CCGGCAACAA AACTTTTAAAG AGAGTGGCGC  
 2451 TGAAGCCGCT GCTTTTGATG ATGGAGACCT AGTGAATGCG TCTATCCCTG  
 2501 TCGGCAATCG GTTAGAAAAA ATCTCCGAGC ATGAAAAAAA TAATTTCCAG  
 2551 ATTTCTCTAG CCTACATTTG TGAATGTGAT CGTAAAAATC CCGGTCGCG  
 2601 TACTTCTCTA ATGGTCAGTG GAGCCCTCTG GACTTCGCTA TGTAAAAACC  
 2651 TCGCAGCACA AGCCTTCTTA GCAAGTGTG GAGGCCATCT GACTCTCTCC  
 2701 CCTCATGTAG AACTCTCTGG GGAAGTGTCT TATGAGCTTC GTGGCTCAGC  
 2751 ACACATCTAC AATGTAGATT GTGGGCTAAG ATACTCATTC TAG

The PSORT algorithm predicts outer membrane (0.927).

40 The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 23A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 23B) and for FACS analysis (Figure 23C). A his-tag protein was also expressed.

The cp6729 protein was also identified in the 2D-PAGE experiment (Cpn0446) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

45 These experiments show that cp6729 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 24

The following *C. pneumoniae* protein (PID 4376849) was expressed <SEQ ID 47; cp6849>:

50 1 MSKLIRRVVT VLALTSMAAC FASGGIEBAV AESLITRIVA SAETKPAVP  
 51 MTAKKVRLVR RNKQPVGEKXS RGAFCDKREY PCEBGRQPV EAQGESCYGR  
 101 LYSVKVNDDC NVEIKQSVP E YATVGSYPPI EILAIGKDC VDVVITQQLP  
 151 CEAEFVSSDP EPTPTSDGKL VWKIDRLGAG DKCKITVWVK PLKBGCCFTA  
 201 ATVCACPELR SYTKCGQPAI CIKQSGPDCA CLRCFVCYKI EVVNTGSAIA  
 251 RNVTVDNFVP GYSHASGQR VLSFNLGDMR PGDKKVETVE FCPQRRGQIT  
 55 301 NVATVTYCGG HKCSANVTIV VNEPCVQVNI SGADMSVCK PVEYSISVSN  
 351 PGDLVLHDEV IQDTLPSGVV VLEAPGGETC CNKVMVRIKE MCPGETLQFK

401 LVVKAQVPR FTRQVAVTSE SNGGCTCTSCA ETTTHWKGIA ATHMCVLDTN  
 451 DFICVGENFV TRICVTRNGS AEDTNVSLIL KFSKELQFIA SSGPTKGTIS  
 501 GNTVVDALPD KLGSKEVVEF SVTLKGIAFG DARGEAILSS DTLTSPVSDT  
 551 ENTHVY\*

# 5 A predicted signal peptide is highlighted.

The cp6849 nucleotide sequence <SEQ ID 48> is:

1 ATGTCCAAAC TCATCNGACG AGTAGTTACG GTCCCTGGCG TAACGAGTAT  
 51 GGCAGGTGCG TTTGCCAGCG GGGGTATAGA GCGCGCTGTA GCAGAGTCTC  
 101 TGATTACTAA GATCGTCGCT AGTGGCGAAA CAAAGCCAGC ACCTGTTCCT  
 151 ATGACAGCGA AGAAGGTTAG ACTGTCCGCT AGAATAAACC AACCAAGTGA  
 201 ACAAAAGAGC CGTGGTGCTT TTTGTGATAA AGAATTTTAT CCCGTGAAG  
 251 AGGACGATG TCACCTGCTA GAGGCTCAGC AAGAGTCTTG CTACGGAAGA  
 301 TTGTATTCTG TAAAAGTAAA CGATGATTGC AACGTAGAAA TTTCCAGTC  
 351 CGTTCACGAA TACGCTACTG TAGGATCTCC TTACCCCTATT GAAATCCTTG  
 401 CTATAGGCAA AAAAGATTGT GTTGATGTTG TGATTACACA ACAGCTACCT  
 451 TGGGAAGCTG AATTCGTAGC CAGTAGTCCA GAAACAACCT CTACAAGTGA  
 501 TGGGAATATA TTCTGGAAAA TCGATCGCCT GGGTCGAGGA GATAAATGCA  
 551 AAATTACTGT ATCGGTAAAA CCTCTTAAAG AAGGTTGCTG CTTCACAGCT  
 601 GCTACTGTAT GTGCTTGCCC AGAGCTCCGT TCTTATACTA AATGCGGTCA  
 651 ACCAGCCATT TGTATTAAGC AAGAAGGACC TGACTGTGCT TGCCATAAGT  
 701 GCGCTGTATG TCACAAAATC GAAAGTAGTA ACACAGGATC TGCTATTGCC  
 751 CGTAACGTAA CTGTAGATAA TCCTGTTCCC GATGGCTATT CTCTATGCATC  
 801 TGGTCAAAGA GTTCTCTCTT TTAACCTAGG AGACATGAGA CCTGGCGATA  
 851 AAAAGGTATT TACAGTTGAG TTCTGCCCTC AAGAAGAGG TCAATCACT  
 901 AACGTTGCTA CTGTAACCTA CTGCGGTGGA CACAAATGTT CTGCAATGTT  
 951 AACTACAGTT GTTAAAGAGC CTTGTGTACA AGTAAATATC TCTGGTGGTG  
 1001 ATTGGTCTTA CGTATGTAAA CCTGTGGAGT ACTCTATCTC AGTATCGAAT  
 1051 CCTGGAGACT TGGTCTCTCA TGATGTGCTG ATCCAAGATA CACTCCCTTC  
 1101 TGGTGTTACA TACTCTGAAG CTCCTGGTGG AGAGATCTCG TGATAAAGG  
 1151 TTGTTTGGCG TATTAAAGAA ATGTGCCCAG GAGAAACCTC CCAGTTTAAA  
 1201 CTTGTAGTGA AAGCTCAAGT TCCTGGAAGA TTCAACAACT AAGTTGCAAT  
 1251 AACTAGTGAG TCTAACTGCG GAACATGTAC ATCTTCGCGA GAAACACAA  
 1301 CACATTGGAA AGGCTTTGCA GCTACCCATA TGTGCGTATT AGACACAAAT  
 1351 GATCCTATCT GTGTAGGAGA AAATACTGTC TATCCTATCT GTGTAACTAA  
 1401 CCGTGGTTCT GCTGAAGATA CTAACGTATC TTTAATCTTG AAGTTCTCAA  
 1451 AAGAACCTCA GCCAATAGCT TCTTCAGGTC CAACTAAGG AACGATTCCA  
 1501 GGTAATACOG TTGTTTTCGA CGCTTTACCT AAACCTCGGT CTAAGGATC  
 1551 TGTAAGGTTT TCTGTTCCTT TGAAGGATAT TGCTCCCGGA GATGCTCGCG  
 1601 GCGAAGCTAT TCTTCTCTCT GATACACTGA CTTCACCAGT ATCAGACACA  
 1651 GAAATACCC ACGTGTATTA A

The PSORT algorithm predicts periplasmic space (0.93).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 24A, and also as a his-tag protein. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 24B) and for FACS analysis (Figure 24C).

# 45 The cp6849 protein was also identified in the 2D-PAGE experiment (Cpn0557).

These experiments show that cp6849 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

## Example 25

The following *C. pneumoniae* protein (PID 4376273) was expressed <SEQ ID 49; cp6273>:

50 1 MGLPHLTLEF LLLCSLPISL VAKFPESVGH KILYISTQST QQALATYLEA  
 51 LDAYGDHDFP VLRKIGEDYL KQSIHSDPD TRKSTIIGAG LAGSSEALDV  
 101 LSQAMETADP LQQLVLVSAV SGHLGKTSDD LLFKALASPY FVIRLEAAYR  
 151 LANLKNKTVI DILHSPHKL PEEIQCLSAI TPLRLETES DAVIRDLLEA  
 201 KSAIRSATA LQIGEFQQR PLPTLRNLIT SASPDQBEI LYAGKIKDGG



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251 QSYYNKKQL QRPDQVDTLA AQAALIALGK EEDALFVIKK QALEERPRAL  
 301 YALRHLPKRI GIPIALPIPL KTKNSEAKLN VALALLELCG DTPKLEYIT  
 351 ERLVQPHYNE TLALSPSKGR TLQNKWRVIN IVQPDQPERE RLSTTRGLE  
 401 EQIITFLFRL PKRAYLPCY KLIASQRTQL ATTAISPLSH TSHQALDLI  
 451 PQAAKLPGEPI IIRAYADLAI YNLTKDPEKK RSLHDYAKKL IQETLFEVDT  
 501 ENQRPHSPMP YLRVQVTFES RTKIMLDILE TLATSKSSSD IRLLIQIMTE  
 551 GDARNFPVLA GLIYKIVB\*

A predicted signal peptide is highlighted.

The cp6273 nucleotide sequence <SEQ ID 50> is:

10 1 ATGGGACTAT TCCATCTAAC TCTCTTTGGA CTTTTFATTGT GTAGTCTTCC  
 51 CATTTCTCTT GTTGCTAAAT TCCTTGAGTC TGTAGGTCAT AAGATCCCTT  
 101 ATATAAGTAC GCAATCTACA CAGCAGGCCT TAGCACAATA TCTGGAAGCT  
 151 CTAGATGCCT ACGGTGATCA TGACTTCTTC GTTTTAAAGAA AANTCGGAGA  
 201 AGACTATCTC AAGCAAAGCA TCCACTCTCT AGATCCGCAA ACTGAAAAAA  
 251 GCACCATCAT TGGAGCAGGC CTGGCGGGAT CTTCAGAAGC CTTGGACGTG  
 301 CTCCTCCCAAG CTATGGAAAC TGCAGACCCC CTGCAGCAGC TACTGGTTTT  
 351 ATCGGCAGTC TCAGGACATC TTGGGAAAAC TTCTGACGAC TTACTGTTTA  
 401 AAGCTTTAGC ATCTCCCTAT CCTGTCACTC CCTTAGAAGC CGCCTATAGA  
 451 CTTGCTAATT TGAAGAACAC TAAAGTCATT GATCATCTAC ATCTTTTCAT  
 501 TCATTAAGCTT CCCGAAGAAA TCCAATGCCT ATCTGCGGCA ATATCTCTAC  
 551 GCTTGGAGAC TGAAGAATCT GATGCTTATA TTGCGGATCT CTTAGCTGCC  
 601 AAGAAAAAGCG CGATTGCGAG TGCACACAGT TTGCGAGTCC GAGAATACCA  
 651 ACAAATAACGC TTCTTCCGCA CACTTAGGAA TTGCTAAACG AGTGGCTCTC  
 701 CTCGAAGTCA AGAAGCTATT CTTTATGCTT TAGGGAAGCT TAAGGATGCT  
 751 CAGAGCTACT ACAATATAAA AAGCAATTG CAGAAGCCTG ATGTGGATGT  
 801 CACTTTAGCA GCAGCTCAAG CTTTAATTGC TTTGGGGAAA GAAGAGGACG  
 851 CTCCTCCCGT GATAAABAAG CAAGCACTTG AGGAGCGGCC TCGAGCCGTG  
 901 TATGCCCTAC GGCATCTACC CTCTGAGATA GGGATTCGGA TTGCCCTGCC  
 951 GATATTCCCTA AAAACTAAGA ACAGCGAAGC CAAGTTGAAT GTAGCTTTAG  
 1001 CTCCTCTAGA GTTAGGGTGT GACACCCCTA AACTACTTGA ATACATTACC  
 1051 GAAAGGCTTG TCCAACACA TTATAATGAG ACTCTAGCCT TGAGTTTCTC  
 1101 TAAGGGGCGT ACTTTACAAA ATTGAAGCG GGTGAACATC ATAGTCCCTC  
 1151 AAGATCCCCA GGAGAGGAAA AGGTGTCTCT CCACAACCCG AGGCTTTGAA  
 1201 GAGCAGATCC TTACGTTTCT CTTCGCCCTA CCTAAGAAGG CTTACTCTCC  
 1251 CTGTATTATAT AAGCTTTTGG CGAGTCAGAA AACTCAGCTT GCCACTACTG  
 1301 CGATTCTTPT TTTAAGTCAAC ACCTCACAAT AGGAAGCCCT AGATCTACTT  
 1351 TTCCAAGCTG CGAAGCTTCC TGGAGAACCT ATCATCCGCG CCTATGCAGA  
 1401 TCTTGTCTATT TATAATCTCA CCAAGATCC TGAAAAAAA CGTTCTCTCC  
 1451 ATGATTATGC AAAAAAGCTA ATTCAGGAAA CCTTGTATT TTGTGACACG  
 1501 GAAAAACAAA GACCCCATCC CAGCATGCCC TATCTACGTT ATCAGGTACC  
 1551 CCCAAGAAGC GGTACGAAAG TCATGTTGGA TATTCAGGAC ACACATGCCA  
 1601 CCTCGAAGTC TTCCGAAGAT ATCCGTTTAT TGATACAACG GATGACGGAA  
 1651 GAGATGCAA AAAATTTCCC AGTCTTGA CA GCTTACTCA TAAAAATTGT  
 1701 GGAGTAA

45 The PSORT algorithm predicts a periplasmic location (0.922).

The protein was expressed in *E. coli* and purified as a his-tag product and as a GST-fusion product, as shown in Figure 25A. The recombinant GST-fusion was used to immunise mice, whose sera were used in a Western blot (Figure 25B) and for FACS analysis (Figure 25C).

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonia.

These experiments show that cp6273 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 26

The following *C. pneumoniae* protein (PID 4376735) was expressed <SEQ ID 51; cp6735>:

-68-

|    |     |                   |                   |            |             |            |
|----|-----|-------------------|-------------------|------------|-------------|------------|
|    | 1   | <b>MTILRNFLTC</b> | <b>SALFLALFAA</b> | AQVYVLHESD | GYNGAINNKS  | LEPKITCYPE |
|    | 51  | GTSYIFLDDV        | RISNVKHDQE        | DAGVFINRSG | NLFFPMGNRCN | FTFHNLMWTE |
|    | 101 | FGAATSNRVG        | DTITLTLNFS        | YLAPTSAPLL | PQGQGAITYSL | GSUMIENSRE |
| 5  | 151 | VTFQGNYSW         | SGAAIYTFYL        | LGSKASRPSV | NLSGNRYLVF  | RDVVSQGYGL |
|    | 201 | AISTHNLTLT        | TRGPSCFENN        | HAYHDVNSNG | GAIAIAPGGS  | TSISVKSDDL |
|    | 251 | IFKGNFAQD         | GNTIHNSIHL        | QSGAQPKNLR | AVSBSGVVYF  | DFISHSESHK |
|    | 301 | ITDLVINAPE        | GKSTYBGTIS        | FSGLCLDEME | VCARNLITST  | LQVITLAGGT |
|    | 351 | LSLSDGVTLQ        | LHSFKQEAAS        | TLTMSPTTLL | LCSGDARVQN  | LHLITLADTN |
| 10 | 401 | FVFPVTRABD        | KDALVSLSKL        | KVAFBAYWSV | YDFPQFKEAF  | TIPLLELQDP |
|    | 451 | SFDSLILGET        | TLERTQVITE        | NDAVRGFWSL | SNBESYFSLD  | KDRRITPTTK |
|    | 501 | TVFLTNWFEI        | TSTP*             |            |             |            |

A predicted signal peptide is highlighted.

The cp6735 nucleotide sequence <SEQ ID 52> is:

|    |      |             |             |            |             |             |
|----|------|-------------|-------------|------------|-------------|-------------|
|    | 1    | ATGACCATAC  | FTCGAAATTT  | TCTTACCTGC | TCGGCTTTAT  | TCCTCGCTCT  |
|    | 51   | CCCTCGACGA  | GCACAAAGTTG | TATATCTTCA | TGAAAGTGAT  | GGTATAAACG  |
|    | 101  | GTGCTATCAA  | TAATAAAAGC  | TAGAAACCTA | AAATFACCTG  | TTATCCAGAA  |
|    | 151  | GGAACTCTCT  | ACATCTTTCT  | AGATGACGTG | AGGATTTCCA  | ACGTTAAGCA  |
|    | 201  | TGATCAAGAA  | GATGCTGGGG  | TTTTTATATA | TCGATCTGGG  | AATCTTTTTT  |
|    | 251  | TCATGGGCAA  | CCGTTGCAAC  | TTCACTTTTC | ACAACCTTAT  | GACCGAGGT   |
| 20 | 301  | TTTGGGCGTG  | CCATTTCSAA  | CCGCGTTGGA | GACACCACCT  | TCACCTCTCT  |
|    | 351  | TAATTTTCTT  | TACTTAGCTG  | TCACCTCAGC | ACCTCTACTA  | CCTCAAGGAC  |
|    | 401  | AAGGAGCGAT  | TTATAGTCTT  | GGTTCGCTGA | TGATCGAATA  | TAGTAGGAGAA |
|    | 451  | GTGACTTTCT  | GTGGGAACCT  | CTCTTCGTGG | AGTGGAGCTG  | CGATTTATAC  |
|    | 501  | TCCCTACCTT  | TTAGGTTCTA  | AGGCGAGTCG | TCCCTCAGTA  | AATCTCAGCG  |
| 25 | 551  | GGAAACCGCTA | CCTGGTGTIT  | AGAGACARTG | TGAGCGAAGT  | TTAATGGCGG  |
|    | 601  | GCCATATCTA  | CCCAACATCT  | CACACTCAGC | ACTCGGAGAC  | CTTCGTGTIT  |
|    | 651  | TGAAAATAAT  | CATGCTTATC  | ATGACGTGAA | TAGTAAATGGA | GGAGCCATTG  |
|    | 701  | CCATTCCTCC  | TGGAGGATCG  | ATCTCTATAT | CCGTGAAAGC  | CGAGATCTCT  |
|    | 751  | ATCTTCBAAG  | GAATACACAG  | ATCACACAGC | GGAAATACAA  | TACACAACTC  |
| 30 | 801  | CATCCATCTG  | CAATCTGGAG  | CACAGTTTAA | GAACTACGTT  | GCTGTTTACG  |
|    | 851  | AAATCCGGAT  | TTATTTCTAT  | GATCTCTATA | GCCATACGGA  | GTCGCATATA  |
|    | 901  | ATTACAGATC  | TTGTAAATCAA | TGCTCCTGAA | GGAAAGGAAA  | CTTATGAAGG  |
|    | 951  | AACAAATPAG  | TTCTCAGGAC  | TATGCTCTGA | TGATCATGAA  | GTTTGTGCGG  |
|    | 1001 | AAATCTCTAC  | TCGCACANTC  | CTACAAGATG | TCACATTAGC  | AGGAGGAACT  |
|    | 1051 | CTCTCTCTAT  | CGGATGGGGT  | TACCTTTGCA | CTGCATTTCT  | TTAAGCAGGA  |
| 35 | 1101 | AGCAAGCTCT  | ACGCTTACTA  | TGCTCTCCAG | AACCACTCTC  | CTCTGCTCAG  |
|    | 1151 | GAGATGCTCG  | GGTTTCAGANT | CTGCACATCC | TGATTTGAAGA | TACCGACAC   |
|    | 1201 | TTTGTTCCTG  | TAGGATTTCC  | CGCCAGGAGC | AAGGATGCTC  | TTGTCTCATT  |
|    | 1251 | AGAAAACTTT  | AAAGTTCGCT  | TTGAGGCTTA | TTGTCTCGTC  | TATGACTTTC  |
|    | 1301 | CTCAATTTAA  | GGAAGCTTTT  | ACGATTTCTC | TTCTTTGAATC | TCTAGGCGCT  |
|    | 1351 | TCTTTTGACA  | GTCTTCTCTC  | AGGGGAGACC | ACTTTTGAGAA | GAAACCAGGT  |
|    | 1401 | CACAAACAG   | AATGACGCCG  | TTCCAGGTTT | CTGTGTCCTA  | AGCTGGGAAG  |
|    | 1451 | AGTACCCCTC  | TTCTCTGGAT  | AAAGACAGAA | GGATCACACC  | AACTAAGAAA  |
|    | 1501 | ACTGTTTCC   | TCACTTGGAA  | TCCTGAGATC | ACTTCTACGC  | CATAA       |

45 The PSORT algorithm predicts an outer membrane location (0.922).

The protein was expressed in *E. coli* and purified as a his-tag product and as a GST-fusion product, as shown in Figure 26A. The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 26B).

These experiments show that cp6735 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 27

The following *C. pneumoniae* protein (PID 4376784) was expressed <SEQ ID 53; cp6784>:

|    |     |                   |                   |                  |            |            |
|----|-----|-------------------|-------------------|------------------|------------|------------|
|    | 1   | <b>MNRKRKRVVV</b> | <b>ALFAMTALIS</b> | <b>VGCCPWQAK</b> | SRCSIDKIYP | VVNRLLVEVG |
|    | 51  | LPEAENVEDL        | IESSAMVLT         | PEERFSGELV       | SIQCVKDEHA | FYNDLSLLHM |
| 55 | 101 | TQAVPSYAT         | YDCAVVFQGG        | LPALRQRLDP       | LVREWRQGRV | FKYIVFLCGE |
|    | 151 | RGRYQSISEQ        | RHFFDSRYNP        | PPTVENWESG       | NRVTFESSE  | IAPVVMQML  |

201 LPRAWRSDTS GVRVTFLLAK FEENRVVANR KDTLLLFRRSY QEAFFGRVLF  
 251 VSSQPFILGLD ACRVGGFFKG BSYDLAGPGF AQGVLYRHYA PRICLHTLAE  
 301 WLKETNGCLN ISEGCFG\*

A predicted signal peptide is highlighted.

5 The cp6784 nucleotide sequence <SEQ ID 54> is:

1 ATGAATAGAA GAAAGCAAG ATGGGTAGTG GCATTGTTTCG CAATGACGGC  
 51 GCTCAITTCCT GTTGGGTGT TGCCTTGGTC ACAAGCGAAA TCAAGATGTT  
 101 CTATTGATAA GTATATTCCCT GTAGTCGAAT GTTTACTAGA AGTTTGGGA  
 151 CTCTCTGAAG CTGAGAATGT TGAGGATTTA ATCGAGTCCT CGTCTGCTTG  
 201 GGCTAGTACT CCTGAAGAAC GTTTTCTCTGG AGAGTTAGTC TCTATCTGTC  
 251 AGGTTAAAGA TGAGCAFGCT TTCTATAACG ATTTGTCCTTT ATTACATATG  
 301 ACTCAGGCTG TGCCCTTCGTA TTCTGCAACG TAGTATTGTG CTGTAGTATT  
 351 TGCGCGGCTT TTGCCAGCGC TACGTCAAGC CTAGATTTT TTGGTGCAGG  
 401 AGTGGCAGCG TGCGCTGCGC TTTAAGAAA TCGTTTCTCT ATGTGGAGAG  
 451 CGAGGGCGCT ATCAGTCTAT TGAAGAACAA GAGCATTTCT TTGATCTCG  
 501 GTACAATCCT TTCCCTACTG AAGAGAACTG GGAATCTGTT AACCGATT  
 551 CTCCCTCTTC TGAAGAAGAG ATTGCCAAAT TTGTTGGAT GCMAATGCTT  
 601 TTACCTAGAG CATGGCGAGA TAGTACTCTA GGAATCAGAG TGACATTTCT  
 651 TCTAGCAAGC CAGAGAGAAA ATCGTGTGGT TGCGAATCGT AAGCACACTG  
 701 TACTTTTATT CCGTCTCTAT CAAGAAGCGT TTCCGGGACG CGTGTATT  
 751 GTAAGTAGTC AACCTTTTAT CGGTTTAGAT GCTTGCAAGG TCGGCGATT  
 801 TTTCAAAGGG GAAAGCTATG ATCTTGCTGG ACCTGGAATT GCTCAAGGAG  
 851 TCTTGAAGTA TCATTGGGCT CCAAGGATTT GTCTACATAC TTTAGCGGAA  
 901 TGGTTAAAGG AAACGAACGG CTGCTTAAAT ATTTCAGAGG GTTGTTTTGG  
 951 ATGA

The PSORT algorithm predicts a periplasmic location (0.894).

The protein was expressed in *E. coli* and purified as a his-tag product and as a GST-fusion product, as shown in Figure 27A. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 27B). The GST-fusion product was used for FACS analysis (Figure 27C).

30 The cp6784 protein was also identified in the 2D-PAGE experiment (Cpn0498).

These experiments show that cp6784 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 28

The following *C. pneumoniae* protein (PID 4376960) was expressed <SEQ ID 55; cp6960>:

35 1 MNRRWNLVLA TVALALSVA **CDVR**SKDKDK DQSLVVEYKD NKDINDIELS  
 51 DNQKLSEFTFG HLLARQLRKS EDMFFDIAEV AKGLQAEIVC XSAPLAEYEV  
 101 BEKMAEIVQKL VPEKSKENL SLAEKFLKEN SKNAGVVEVQ PSKLQYKLIK  
 151 EGAGKAISGK PSALLHYKGS FINGQVSSS EGNNEPILP LGQITPGFAL  
 201 GMQGMKEGET RVLYIHPDLA YGTAGQLPPN SLLIFEINLI QASADBVAAV  
 251 PQEGNQGE\*

A predicted signal peptide is highlighted.

The cp6960 nucleotide sequence <SEQ ID 56> is:

1 ATGACACAGAC GGTGGAATTT AGTTTATGCA ACAGTAGTCT TGGCACTCTC  
 51 CGTCGCTTCT TGTGACGTAC GGTCTAAGGA TAAAGACAAG GATCAGGGGT  
 101 CGTTAGTGGTA ATATAAGATG AACAAAGATA CCAATGACAT AGAATTATCC  
 151 GATAATCAAA AGTTATCCAG AACATTGGT CATTTATTATG CAGGCCAATT  
 201 ACGCAAGTCA GAAGATATGT TTTTGTGATAT TGCAGAAAGT GCTAAGGGGT  
 251 TGCAGGCGGA ATTGGTTTGT AAAAGTGCTC CTITTAACAGA AACAGAGTAT  
 301 GAAGAAAAAAA TGGCTGAAGT ACAGAGTTTG GTTTTGTAAA AAAAATCAAA  
 351 AGAAAAATCTT TCATTGGCAG AAAAATCTCT AAAAGAAAAT AGCAGAACCG  
 401 CTGCTGTTGT TGAAGTGCAA CCAAGTAAAT TGCATATCAA AATTATTAAA

5 451 GAAGGTGCAG GGAAGCAAT TTCAGGTAAA CCITCAGCTC TATTGCACCTA  
 501 CAAGGGTTCC TTCACTCAATG GCCAAGTATT TAGCAGTTCA GAAGGCAACA  
 551 ATGAGCCTAT CTTGCTTCCT CTAGGCCAAA CAATTCCTGG TTTTGCTTTA  
 601 GGTATGCAGG GCATGAAGA AGGAGAACT CGAGTTCCTT ACATCCATCC  
 651 TGATCTTGCT TACGGAACCG CAGGAAACT TCCTCCAAC TCTTTATTA  
 701 TTTTGAAT TAACTTGATT CAGGCTTCAG CAGATGAAT TGCTGCTGTA  
 751 CCCCAAGAAG GAAATCAAG TGAATGA

The PSORT algorithm predicts periplasmic space location (0.930).

10 The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product, as shown in Figure 28A. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 28B) and for FACS analysis (Figure 28C).

The cp6960 protein was also identified in the 2D-PAGE experiment.

These experiments show that cp6960 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### 15 Example 29

The following *C.pneumoniae* protein (PID 4376968) was expressed <SEQ ID 57; cp6968>:

20 1 MKFLLYVPLL LVLVSTGCDA KPVSEFPFSG KLSTQRFEPQ HSAEEVFSQG  
 51 QEFLLKGNFR KALLCGIIT HHPFRDILRN QAGYLIGVCY FTQDHPDLAD  
 101 KAFASYQLP DAESSEELFQ MKYATQRFQ QGRRKRICRL EGFPKIMNAD  
 151 EDALRIYDEI LTFAPSKDLG AQALYSKAAL LIVKNDLTER KTKLEKLTLQ  
 201 FPLHILSSEA FVRLSEIYLO QAKKSPHNLQ YLHFAKINEE AMKQHPNPD  
 251 LNEVVSANVG AMREHYARGL YATGRFYEKK KKAERANIY RTAITNVPDT  
 301 LLVAKCQKRL DRISKHTS\*

A predicted signal peptide is highlighted.

25 The cp6968 nucleotide sequence <SEQ ID 58> is:

30 1 ATGAAATTTC TATTATACGT TCCACTTCTT CTTGTTCTCG TATCTACGGG  
 51 GTGCGATGCA AAACCTGTTT CTTTTCAGCC CTTTTCAGGA AAGCTTTCCA  
 101 CCCAGCGTTT TGAGCCTCAG CACTCTGCTG AAGAATATT TTTTCAGGGA  
 151 CAGGAATTCT TAAAAAAGG AAATTTCAGA AAAGCTTTAC TATGCTTTGG  
 201 AATCATTTACG CATCTACTTC CTAGGGACAT CTTGCGTAAT CAAGCAGAGT  
 251 ATCTTTATAGG AGTCTGTAC TTCACGCGAG ATCACCAGCA TTTAGCAGAC  
 301 AAGGCATTTC CATCTTACTT ACAACTTCTT GATGCGGAGT ACTCTGAAGA  
 351 GTTGTCCTAG ATGAAATATG CGATGTCTCA AAGATTTCGT CAAGGGAAGC  
 401 GTAAACGGAT TTGTCGATTG GAGGGCTTCC CAAACTAAT GATGCTGAT  
 35 451 GAAGATGCGC TACGCATTTA TGACGAGATT CTAACAGCGT TTCTAGTAA  
 501 AGACTTAGGA GCTCAGGCC CCTATAGTAA AGCTGCGTTA CTTATGTATA  
 551 AAAACGATCT TACAGAAGCC ACCAAAACCT TAAAAAACT CACGTTACAA  
 601 TTTCTCTTAC ATATTTTATC TTCAGAGGCC TTTGTACGTT TATCGGAAT  
 40 651 CTATTTCACG CAAGCTAAGA AAGAGCCTCA CAATCTCTAA TATCTTCAIT  
 701 TTGCAAGAGCT TAATGAAGAG GCAATGAAGA AGCAGCATCC TAACCATCTT  
 751 CTGAATGAGG TTGTTTCTGC TAATGTTGGA GCTATGCGGG AACATTATGC  
 801 TCGAGGTTTG TATGCCACAG GTCGTTCTTA TGAGAAGAAG AAAAAAGCCG  
 851 AGGCTGCGAA TATCTATTAC CGCATTCGCA TTACAACTA CCCAGACACT  
 901 TTATTAGTGG CTAAATGTCA AAGCGTCTA GATAGAATAT CTAAGCATAC  
 45 951 TTCTCTAA

The PSORT algorithm predicts an inner membrane location (0.790).

The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product, as shown in Figure 29A. The recombinant GST-fusion was used to immunise mice, whose sera were used in a Western blot (Figure 29B) and for FACS analysis (Figure 29C).

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6968 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### 5 Example 30

The following *C.pneumoniae* protein (PID 4376998) was expressed <SEQ ID 59; cp6998>:

```

1  MKKLLKSAALL SAAFPAGSVGS LQALPVGNGPS DPSLLIDGTI WEGAAGDPCD
51 PCATWCDIAIS LRAGFYGDYV FDRILKVDAP KTFMSGAKPT GSAAANYTTA
101 VDRPNPAYNKR HLHDAEWFTN AGFIALNIMD RPDVFTCLGA SNOYIRGNST
151 AFNLVGLFQGV KGTTFVNAHEL PNVLSLNGUV BLVYDTSPFSW SVGARGALWE
201 CGCATLGAEP QYAQSKPKVE ELNVICNVSQ FSVNKPQKV GVAFFLPPTDA
251 GVATATGATKS ATINYNHWQV GASLSYRLNS LVFYLGVQNS RATFDADNIR
301 IAQPKLPTAV LNLTAWNPSL LGNATALSTT DSFSDFMQIV SCQINPKFRS
351 KACGVTVGAT LVDADKWSLT AEARLINERA AHVSGQFRF *
```

15 A predicted signal peptide is highlighted.

The cp6998 nucleotide sequence <SEQ ID 60> is:

```

1  ATGAAAAAAC TCTTAAAGTC GGGCTATTTA TCGCGCCGAT TGCCTGTTTC
51 TGTTCGCTCC TTACAAGCCT TGCCTGTAGG GAACCCCTTC GATCCAAAGCT
101 TATTAAATGA TGGTATCAATA TGGGAAGGTG CTCGACGAGA TCCTTCGCGAT
151 CTTTGGGCTA CTGGTGTGCGA CGCTATTAGC TTACGTSGTG GATTTTACGG
201 AGACTATGTT TTGCAACGTA CTTTAAAGGT AGATGCAACT AAACAATTTT
251 CTATGGGAGC CAAGCCTACT GATCCGCTG CTGCAAACTA TACTATCGCC
301 GTAGATGAGC CTAACCCGGC CTAACAATAAG CATTTACACG ATGCAGAGTG
351 GTTCACTAAT CGAGGCTTCA TTGCCTTAAA CATTTGAGAT CGCTTTGATG
401 TTTTCTGTAC TTTAGGAGCT TCTAATGGTT ACAATTAGAG AAACCTCACA
451 CGCTTCAATC TCGTTGCTTT ATTCCGAGTT AAAGGTACTA CTGTAAGATC
501 AAATGAACATA CCAAACGTTT CTTTAAGTAA CGGAGTTGTT GAACCTTTACA
551 CAGACACCTC TTTCTCTTGG AGCGTAGGCG CTCGTGGAGC CTTATGGGAA
601 TGGCGTTGTG CAACCTTGGG AGCTGAATTC CAATATGCAC AGTCCAAACC
651 TAAAGTTGAA GAACCTTAATG TGATCTGTAA CGTATCGCAA TTCTCTGTAA
701 ACAAAACCAA GGCCTATAAA GCGCTTGCTT TCCCTTGCCG AACAGACGCT
751 GCGGTAGCAA CAGCTACTGG AACAAAGTCT GCGACCATCA ATTATCATGA
801 ATGGCAAGTA GGAGCCTCTC TATCTTACAG ACTAAACTCT TTAGTGCCAT
851 ACATTGGAGT ACAATGGTCT CGAGCAACTT TTGATGCTGA TAACAATCCG
901 ATTGCTCAGC CAAACCTACC TACAGCTGTT TTAACCTTAA CTGCAATGAA
951 CCCTTCTTTA CTAGGAAATG CCACAGCAAT GTCTACACTG GATTCGPTCT
1001 CAGACTTCAT GCAAATGTGT TCCTCTCAGA TCAACAAGTT TAAATCTAGA
1051 AAAGCTTTGT GAGTTACTGT AGGAGCTACT TTAGTTGATG CTGATAAATG
1101 GTCACTTACT CGAGAAGCTC GTTTAATTAA CGAGAGAGCT GCTCACGTAT
1151 CTGCTCAGTT CAGATCTCTAA
```

The PSORT algorithm predicts an outer membrane location (0.707).

The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 30A) and as a his-tag product. The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 30B) and for FACS analysis (Figure 30C).

45 The cp6998 protein was also identified in the 2D-PAGE experiment (Cpn0695) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that ep6998 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

## Example 31

The following *C.pneumoniae* protein (FID 4377102) was expressed <SEQ ID 61; cp7102>:

```

1  MKHTFTKRVL FFFFLVFIPI LLLNLNVVGF FSTSAAKANL VQVLHTRATN
5  51 LSIIEFEKKLT IHKFLFLDRJA NTLALKSVAS PSAEPYAQAY NEMMALSNVD
101 FSLCLIDPFD GSVRTNPGD PFIIRYLKQHP EMKKKLBAAV GKAFLLTIPG
151 KPLLHLVLLV EDVAVSDSTT TSGLLVSPFY MSFLQKDLQF SLHITKGNIC
201 LVNKGVEVLV CAQDSESSFV FSLDLNLPLQ FQARSPSAIE IEKASGILGG
251 ENLITVSINK KRYLGLVLNK IPIQGYTTLV LVFVSDLIQS ALKVPNLNFC
301 FVYLAFLLMW WIFSKINTKL NKPLOELTFC MEAAWRGNHN VRFEPQPVGY
10  351 EFNELGINFN CTTLLLLLSI EKADIDYHSG EKLQLELGIL SLLQSALLPS
401 DPFTFPKVTF SSOHLRRRLQ SGHFNWTVQ DGGDTLLGLII GLAGDIGLPS
451 YLYALSARSL FLAYASSDVS LQKISKDTAD FSKTTEGNE AVVAMTFIKY
501 VEKDRSLELL SLSEGAPTMF LQRGSEFVRL PLETHQALQP GDRLLCLTGG
551 EDILKYFSQL PIEELLKDPL NPLNTENLID SMTMLNNET EHSADGTLPTI
15  601 LSF5*
```

A predicted signal peptide is highlighted.

The ep7102 nucleotide sequence <SEQ ID 62> is:

```

1  ATGAAACATA CCTTTACCAA GCGTGTCTTA TTTTITTTCT TTTTAGTGAT
5  51 TCCCATTCCTC CTACTCCTCA ATCTTATGGT CGTAGGTTTF TTCTCATTTT
20  101 CTGCGCGTAA AGCAATTATA GTACAGGTCC TCCATACCGC TGCTACGAAC
151 TTAGTATAG AATTGCAAAA AAACTGACG ACATACACAGC TTTTCTCGA
201 TAGACTTGCC AACACATTAG CTTTAAATC CTATGCATCT CCTTCTGAG
251 AGCCCTATGC ACAGGCATAC AATGAGATGA TGGCACTCTC CATACACAGC
301 TTTTTCCTTAT GCCTTATAGA TCCCTTTGAT GGATCTGTAA GGACGAAAAA
25  351 TCCTGGAGAC CCTTTCATTC GCTATCTAAA ACAGCATCTT GAATGGAAGA
401 AAAAGCTATC CGCAGCTGTA GGGAAAGCCT TTTTATTGAC CATTCGAGT
451 AAACCACTTT TACATTTATCT TATTTCTAGT GAAGATGTGC CATCTTGGGA
501 TTCTACAACG ACTPCAGGAC TGCTGTAGAG TTTCATGCC ATGTCTTTT
30  551 TACAGAAGA TTTATTCCAA TCCTTACACA TCACCAGAGG AAATATCTGC
601 CTGTATAATA AGTATGGCGA GGTCTCTTTC TGTCCTCAGG ACAGTGAATC
651 TTCTTTTGTA TTTTCTCTAG ATCTCCTCTA TTTACGCCAA TCTCAAGCAA
701 GAAGCCCTCT TGCCATAGAA ATTGAGAAAG CTTCTGGAA TCTTGGTGGG
751 GAGAACCTAA TCACAGTGAG TATCAACAAG AAACGCTACC TAGGATTTGT
35  801 ACTGAATAAA ATTCTTATCC AAGGGACCTA CACTCTATCT TTAGTTCCAG
851 TTCTCTAGTCT CATCCAATCC GCTTGTAAAG TTCTCTCAA TATTGTTT
901 TTCTATGTAC TTGCTTTCTT CCTCATGTGG TGGATTTTCT CTAAGATCAA
951 CACCAAACTT AACAAAGCTC TTCAAGAACT GACCTTCTGT ATGGAAGCTG
1001 COTGGCGAGG AAACCAATAC GTGAGGTTTG AACCCAGGCC TTACGGTTAT
40  1051 GAATTCATAG AACTAGAGAAA PATTTTCAAT TGCACTCTCC TACTCTTATT
1101 GAATTCATTG GAGAAAGCAG ATATCGATTA CCATTACAGC GAAAAATTAC
1151 AAAAAGAAAT AGGGATTTTA TCTTCACTAC AAAGTGCGTT ACTAAGTCCG
1201 GATTTCCCTTA CGTTCCTTAA AGTATACCTT AGTPTCCAAC ATCTCGGAG
45  1251 AAGGCAACTT TCGGATCAAT TTAATGGTGT GACAGTTCAA GATGGTGGCG
1301 ATACCTCTTT AGGGAATCAT GGGCTCGCTG GCGAATTTGG TCTTCTCTCC
1351 TATCTCTATG CTTTATCCGC ACGGAGTCTT TTTCTTACCT ATGCTCTCTC
1401 GGAGCTTTTCG TTACAAAAAA TCAGCAAGGA TACTCCGCAG AGCTTCTCAA
1451 AANCAACAGA AGGCAATGAG GCTGTATGTT CTATGACTTT CATTAATAAT
1501 GTAGAAAAAG CTGATGCTTC AGAGCTCCTC TCGTATTAAG AGGAGCTCC
50  1551 TACCATTGTTT TCAACAAGAG GAAATATCTT CGTATCGTTC CCTTAGAGAA
1601 CTACCAACGC TCTACAGAGT GGAGATCGGT TGATCTGCTC CACTGGAGGA
1651 GAAGCATCCG TCAAGTACTT TTCTCAGCTT CCTATTGAGT AGCTCTTAAA
1701 AGATCCCTTTA AACCCCTTAA ATACAGAGAA TCTTATPGAT TCTCTAACCA
1751 TGATGTAAAA CAACGAATCC GAACATCTCG CAGATGGAAC TCTGACCTTC
1801 CTTTCAATTTT CATAA
```

55 The PSORT algorithm predicts an inner membrane location (0.338).

The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product. The purified GST-fusion product is shown in Figure 31A. The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot and for FACS analysis (Figure 31B).

These experiments show that cp7102 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 32

The following *C.pneumoniae* protein (PID 4377106) was expressed <SEQ ID 63; cp7106>:

```

5      1 MKDLGTLGGT SSTAKTVSPD QKVMGRSQI ADQSNHAFMC HTDFSSNNIVL
      51 FDLDMTYKTL RENGROI NSI FNLQNMMLQR ASDHEPFPFG RSNIALGAGL
      101 VYNALQNLPL NLAAQYFGIA YKIRPKYRLG VFLDHPFSSG VPMNFVNSHN
      151 RIMNGAFTGW QDSALGSSV KVSFGYKQK ATTTREOLEN TEAGSGESHP
      201 EGVAQIEGR YOKSLQGHVR VQPFGLQLQV HITRKHYTEN AVQFFVHYDP
10     251 IDYSTGVVYL GIGSHIALVD SLFVGTIRMG EQNPAHTDR FSGSLASIGN
      301 FVFEKLDVTH TRAFARMRVN YELFYLQSLN LILRVNQQL QGVMGFSSDL
      351 RYALGF*

```

The cp7106 nucleotide sequence <SEQ ID 64> is:

```

15     1 ATGAAGATT TGGGGACTCT TGGGGGTACC TCTTCTACAG CAAAAACAGT
      51 GTCCCCAGAT GGTAAAGTGA TCATGGGTAG ATCACAAATT GCTGATGGCA
      101 GTTGCCACGC ATTTATGSGT CATACGGATT TCTCTCTAA TAATGTACTC
      151 TTTGATCTCG ATAATACGTA TAAACCTCTA AGAGAAAATG GCCGTGAGCT
      201 AAAATCCATA TTCAACCTAC AAAATATGAT GTTACAGAGA GCCTCAGATC
      251 ATGAGTTTAC AGAGTTTGGG AGGAGTAAAC TCGCTCTTGG TGCGGCGCTT
      301 TATGTGAATG CCTTGCAGAA TCCCTCTAGC AATTTTAGCAG CACAATATTT
      351 TGGAAATCGA TACAAAATAC GTCCCTAAAT TCGTTTGGGG GTGTTTTTGG
      401 ACCATAATTT CAGCTCCACG GTCCCTAAAT ATTTTAACTG AAGCCACAT
      451 AGACTCTGGA TGGAGGCCCT TATTGGATGG CAGGATTCGT ATGCTCTAGG
      501 ATCTAGTGTC AAGGTGTCCT TCGGATATGG AAAACAAAAA GCCACGATT
25     551 CAAGAGAGCA ATTAGAGAA ATACAGAGCG GGAGTGGGGA GAGCCATTTT
      601 GAAGGGGTCG CTGCTCAGAT AGAAGGGGCG TATGTGAAGA GCCTCGGAGG
      651 ACATGTACAG GTCCAGCCTT TCTAGGAGT GCAGTTTGTG CACATTACAA
      701 GGAAGAAATA TACCAGAAAT GCAGTGCAT TCTCTGTACA CTATGATCTC
      751 ATAGACTATT CTACAGGTGT AGTGTATTTA GGAATTGGAT CTCATATTGC
30     801 ACTTGTGATG TCTTTACATG TAGGCACACG CATGGGAATG GAGCAAAATC
      851 TTGCAAGCCA TACGAGACAG TTTCTCAGAT CTATAGCGTC TATTGAAATC
      901 TTTGTGTTTG AAAAGCTTGA TGTGACTCAC ACAAGGCGAT TTGCGAAAT
      951 GCGTGTCAAC TATGAGCTTC CCTATCTACA GTCTCTGAAT CTTATTCTAC
100    1001 GAGTTAATCA ACAGCTCTCA CAGGGGTTA TGGGATTTTC CAGTGATCTT
35     1051 AGGTATGCGT TAGGATCTCA A

```

The PSORT algorithm predicts a cytoplasmic location (0.224).

The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product. The purified GST-fusion product is shown in Figure 32A. The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 32B) and for FACS analysis (Figure 32C).

This protein also showed very good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp7106 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 33

The following *C.pneumoniae* protein (PID 4377228) was expressed <SEQ ID 65; cp7228>:

```

      1 MTAVALILTSF PSESARSILA RHLITERLAS CVHVFPKQTS TYLMEGKLCB
      51 SREHHIQIKS IDIRFSEICL AIQEFSGYEV FEVLLFPFIN GDRPYLNMWT
      101 ILSYPEKKPL SD*

```

The cp7228 nucleotide sequence <SEQ ID 66> is:

```

1   ATGACTGCTG TTCTTATCT TACATCTTC CTTTCGGAGG AAGTGCTCG
51  CTCCTTAGCT AGACATCTGA TTACAGAGCG TCTTGCTCTC TGTGTGCATG
101 TATTCCCTAA AGGCACATCG ACATATCTAT GGGAGGCGAA GCTATGTGAG
151 TCTGAAGAAC ATCATATACA AATCAATATG ATAGACATAC GCTTCTCGGA
201 AATTGTGCTT GCTATTCTGG AGTCTCTCGG CTATGAGGTT COTGAAGTCT
251 TACTATTTCG TATTGAAAT GGGGATCCGA GGTACTTGAA TTGGTTAAGC
301 ATTCTCAGCT ATCCAGAGAA GCCTCCGCTT TCAGATTAG

```

The PSORT algorithm predicts an inner membrane location (0.040).

The protein was expressed in *E. coli* and purified as a his-tag product and as a GST-fusion product, as shown in Figure 33A (his-tag = left-hand arrow, GST = right-hand arrow). The proteins were used to immunise mice, whose sera were used in a Western blot (Figure 33B) and FACS analysis.

These experiments show that cp7228 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 34

The following *C. pneumoniae* protein (PID 4377170) was expressed <SEQ ID 67; cp1710>:

```

1   MNSKMLKHLR LATLSFSMFF QIVSSPAVYA LGAGNPAAPV LFGVNPQGTG
51  WCAPQLCNSY DLPALAGSL KFGPYGDYVF SSHAHTNPV VITSVTTSGT
101 GTTPTITSTT KNVDPLNNS SISSCVFAT IALQETSPA IPLLDIAFTA
201 RVGGLKQYVR LPLNAYRDPF SNPLNARSEV TDGLLEVQSD YGVIVWGLSLQ
251 WSASIGISTY LNDYVLPYAS VSIQNTSRKA PSDSPTELEK QTNFKFKIR
301 KITNFDVRNV CPGTTCCLSN NFYYSVGRW GYQRAINIS GLQF*

```

A predicted signal peptide is highlighted.

The cp1710 nucleotide sequence <SEQ ID 68> is:

```

1   ATGAATAGCA AGATGCTAA ACATTACGT TTAGCAACCC TTTCCTTCTC
51  TATGTTCTTC GGGATGTGAT CTCTCCCGC AGTATATGCC CTAGGGGCTG
101 GAAACCCGTC AGCTCCAGTA CTCCAGGTG TGAATCCTGA GCAACCGGA
151 TGGTGTGCCT TCCAACTTTC TAATAGTTAC GATCTTTTTC GTGCTCTTGC
201 AGGAAGCCTC AATTTGGGGT TCTATGGAGA TTATGCTCTC TCAGAAAGTG
251 CCCATATTAC CAATGTCCCT GTCATTACCT CCGTTACGAC TTCAGGCACA
301 GGAACAACGC CAACATTAC CTCTACACT AAAAACGTAG ACTTTGATCT
351 TAAACAACGC TCCATCAGCT CGAGCTGTGT TTTTGCAACC ATAGCTCTAC
401 AGGAACATC CCGAGCTGCC ATTCCCTTT TAGATATAGC CTCTACGTCA
451 CGTGTGGAG GACTTAAACA GTACTACGC CTCCCTCTCA ATGCTTACAG
501 AGACTTCACT TCAAATCCTT TAAATGCAGA ATCTGAAGTT ACAGATGTCT
551 TCATTGAAGT CCACTACGAC TATGGAAATG TCTGGGCTCT GAGTTTACAA
601 AAGATATTGT GAAAGATGG AGTGCTTTT GTAGGGGTGA GCGCTGACTA
651 CCGTCAACGT TCCAGTCCCA TCAACTATAT CATCGTTTAC AACAAGGCCA
701 ACCCCGAGAT CTAATTTCGAT GCTACTGATG GAAACCTTAA CTATAAGAA
751 TGGTCTGCAA GCATCGGCAT CTCTACGTAT CTTAATGACT ATGTGCTTCC
801 CTAATGCACT GTAATCTATG GAATACCTTC AAGAAAGCT CCTCTGATA
851 GCTTCACAGA ACTCGAAAG CAATTACGAC ATTTTAAAT TAAAAATCGT
901 AAAATCACAA ACTTCGACAG AGTAAACTTC TGCTTCGGAA CTACCTGCTG
951 CATCTCAAA TACTTCTACT ATAGTGTAGA AGGCCGTGG GGATATCAGC
1001 GTGCTATCAA CATTACGTCA GGTCTGCAGT TTTAG

```

The PSORT algorithm predicts a bacterial outer membrane location (0.936).

The protein was expressed in *E. coli* and purified as a his-tag product and as a GST-fusion product. The purified GST-fusion product is shown in Figure 34A. The GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (34B) and for FACS analysis (34C).



The cp7170 protein was also identified in the 2D-PAGE experiment (Cpn0854).

These experiments show that cp7170 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 35

- 5 The following *C.pneumoniae* protein (PID 4377072) was expressed <SEQ ID 69; cp7072>:

```

1 MDIKKLFCLF LCSSLIAMSP IYKGTGDYER I/LTGINIID RNLSEPTICS
51 KEKLLKKYTKV DFLAPOPYQK VMRMVKNKRQ DNVSLTAYH TNGQIKQYLE
101 CLNINRAYGRY REMHVNGNIK IQAEVIGGIA DLHPAESGWM LFDQ"TFAYN
151 DEGLIEAAIV YEKGLLEGSS VVYHTNGNITW KCEPYHKGVP QGKFLTYTSS
201 GKLLKQBNYQ QGRHGLSIR YSEDESDVL AWEEYHEGRL LKARYLDLDPQT
251 HEIYATIHSG NGIQATYGYK AVIETRAFYR GBFYGVKTRF DMSGTQIVQT
301 YNLLQGAKHG EEPFFYPETG KPKLLLNWHH GLINGIVKTV YPGGTLESCR
351 ELVNKKSLG LTIYYPEGQI MATEBYDNDL LIKGEYFRFG DRHPYSKIDR
401 GCGTAVVFSS AGTITKKIPY QDGKPLLN*

```

- 15 A predicted signal peptide is highlighted.

The cp7072 nucleotide sequence <SEQ ID 70> is:

```

1 ATGGATATAA AAAAAGCTCT TTGCTTATTT CTATGTCTCT CTCTAATTGC
51 CATGAGTCCC ATTTATGGGA AAACAGGTGA CTATGAGAAA CTCACCCCTTA
101 CAGGGATCAA TATCATTTGAT AGAAACGGCC TGTCAGAAAC TATTTGTCTCT
151 AAGAGGAAGC TAAAGAAATA CACCAAGGTA GACTTCTCTG CTCGCCAGCC
201 CTATCAAAGG GTCAATGAGGA TGTATAAAAA CAAACCGGGA GATACAGTTT
251 CTTGTTTAAAC AGCCTATCAC ACTAACGGGC AAATTAAACA GTACCTGGAG
301 TGTCCTCAATA ATCGTGCTTA TGGAGAGTAT CGTGAATGGC ACGTCAACGG
351 GAATATCAAA ATCCAAGCTG AGGTTATCGG AGGTATTGGG GATCTTCAATC
25 401 CCTCAGCAGA GTCTGGCTGG CTATTGATC AAACACATAT TGCTTATAAT
451 GATGAAGGTA TCTTAGAAGC CGCTATCGTC TATGAAAAGG GGCTGCTCGA
501 AGGATCTTCG GTGTATTACC ATACTAATGG GAATATTGGG AAGAGATGTC
551 CCTATCATAA GGGAGTTUCT CAAGGTAAAT TCCTGACATA CACATCTTCG
601 GGGAACTGAC TCAAGGAACA GAATATACCA CAAGGCAAAA GACACGGTCT
30 651 TTCGATTCGC TACAGGGAAG ATTCCGAAGA AGATGTTTAA GCCTGGGAAG
701 AATATCATGA GGGACGACTC CTAAGACGAG AGTATCTTGA TCCTCAAACT
751 CACGAATCT ATGCGACTAT ACACGAAGGG AACGGCATTC AAGCAATCTA
801 CGGCAAGTAT GCCGTATAG AAACTAGGGC ATTTTATCCGA GGGGAACCTT
851 ATGGAAAAGT TACCAGATTG GACAACCTCG GAACAATTTT TGTTCCAAAG
35 901 TATAACCTTT TGCAAGGGCG GAGGACAGGA GAACAATTTT TCTTTTATCC
951 TGAGACAGGG AAACCCCAAGC TGCTCTTTAA TGCGCATGAA GGAATTTTAA
1001 ATGGGATAGT AAACAATTTG TATCCCGGAG GAACCTTAGA AGATTTTAAA
1051 GAATCTGTAA AATCAAAAAA ATCCGGGTTA CTGACCATTT ACTACCCCTA
1101 AGACAGAGTC ATGCGCAGCC AAGAGTATGA TATGATCTTT CTAATTTAAG
40 1151 GAGAGTACTT CCGCCCTGGA GACCTCATC CCGTACTCTAA AATAGATCGT
1201 GGTGTGGGA CTGCAGTATT TTTCTCTGCG GCGGGAACTA TTACTAAAAA
1251 AATCCCCAT CAGGACGGCA AACCTTTGCT CAACATAG

```

The PSORT algorithm predicts a periplasmic location (0.688).

- 15 The protein was expressed in *E.coli* and purified as a his-tag product (Figure 35A) and as a GST-fusion product (Figure 35B). The recombinant his-tag protein was used to immunise mice, whose sera were used in a Western blot (Figure 35C) and for FACS analysis.

These experiments show that cp7072 is a useful immunogen. These properties are not evident from the sequence alone.

### Example 36

- 50 The following *C.pneumoniae* protein (PID 4376879) was expressed <SEQ ID 71; cp6879>:

1 MATPAQKSPT FQDPSTVREL GSNHPVFSPL TLEERGEHMAI ARVQQCGWNH  
 51 TIVKVSLLIL ALLTLTGGGL LVULLPAVPM FICTGLIALG AVIFALGALL  
 101 CLYDSQGLPE ELFPVTEPQQ IQEEDLNRET REVLQPLE VLKDRDARD  
 151 PAVTPQVVDK EKRQLDRQL LRKREELLYR STAHKIDDER YFLLLELLEM  
 201 RSLVADRLVF NRRSYERFVQ GLMTVRSSEG EKSISRLQDL SLGQQTVDQ  
 251 LRSLIDDEQK RCWTALQRIN QSQKDIQRAH DRBASQKACE GTTMDCAERL  
 301 QLEKDLRLQL KSMQWLEMR GTTHQSEKAW RKQNAKLERL QEDRLALNGTA  
 351 FDEQSLFYRE YKEKYLSQL DMGKLLQWV AEKSEKACLE SLVHDVEKQL  
 401 EQKDAHLKKA AANWEREIGK QSQDYEQTC ETRRLSTTIL EYQDSLRAAE  
 451 QVEKDFQBLQ QRYSLQEEK QVEKILLES MNHFADLPFK AQREMAVYKK  
 501 KLADLEGRAA PTEIGKDDDW VLTPDSASLQ KKRLELVREN QELKLALAPK  
 551 SMELTQLVAD AVEAEKEISK LRHEIEBQKE GLRALDKHHA QATKDCAAAPK  
 601 RKCCDLSESL SPVREDAQMR FELVEILQLR QENNAQLRAE VERLEBQEPQ  
 651 G\*

15 The cp6879 nucleotide sequence <SEQ ID 72> is:

1 ATGGCAACAC CCGCTCAAAA ATCCCTTACA TTTCAGATC CTAGTTTGTG  
 51 AAGAGAGCTA GGCAGTAACC ACCCTGTCTT TCCCGCGCTA ACGCTTGAGG  
 101 AAGAGAGGGA GATGGCAATA GCTCGAGTCC AGCAGTGTGG ATGGAATCAT  
 151 ACAATTGTTA AGGTAAGTCT TATTATTCTT GCTCTCTCTA TTATTTTGGG  
 201 GGGAGGATTA CTCGTAGGAT TGCTGCCAGC AGTTCCTATG TTTATTGGAA  
 251 CAGGCTGAT TGCTTTGGGA GCGCTATAT TGTGCTTGGC TTTGATTTTA  
 301 TGCTCTTATG ATTCTCAGGG CCTTCTGAG GAACTCCTC CGGTTCCTGA  
 351 ACCACAACAA ATTCTAGATT AGAATTAAAG AAGCAGAGCC AGAGAAGTTC  
 401 TTGAAGGGAG TCTTTTAGAG GTTCTCTTAA AGGATAGAGA CGTAAGGAC  
 451 CCGCGCGTGC CCCAGGTGCT TGTAGACTGT GAAAGAGCTC TTGGAATGTT  
 501 GGATCGTAAG CTCGCAGCTG AAGAGGAGAT TCTGTATCGC TCGACGCCCC  
 551 ATCTTAAAGA CGAAGAAAGG TATGAGTCTT TGCTGGAGCT CTGGAAATGT  
 601 CGTAGTCTGG TTGCCGATCG CTAGAAATT AACCGTAGAA GTTATGACG  
 651 ATTTGTTCAA GGAATTATGA CAGTTAGACT AGAGGAGGGG GAAAAGAGAG  
 701 TTTCTCGTCT ACAAGATCTA ATCASTTTGC AGCAGCAGAC GGTCAAGAT  
 751 TTAAGGAGTC GGATCGATGA CGAGCAGAAG AGATGCTGGA CGGCTTTACA  
 801 ACGTATTAA CAACTCTCAGA AGGATATACA ACGGCTCAT GATCGCGAGG  
 851 CTTCGACGCG TGCTCTGTAG GGCACAGAGA TGGATTGTGC AGAACGCCAG  
 901 CAACCTGGAGA AGGATTTAAG GAGACAGCTG AATCTATGCG AGGAGTGGAT  
 951 TGAGATGAGG GGCACAATCC ATCAACAAGA GAAGGCTTGG CGTAAGCAGA  
 1001 ATGCGAAATT AGAAGGATTA CAAGAGGATC TGAGACTTAC TGGGATTGCT  
 1051 TTTGACGAAC AATCTCTGTT CTATCGCGAA TATAAAGAGA AATATCTGAG  
 1101 TCAGAAACTA GATATGCAAA AGATTTTACA GGAAGTCAAC GCAGAGAAAA  
 1151 GTGAGAGGCG TTGCTTAGAG AGTCTGTGCC ATGACTATGA GAAGCGGCTC  
 1201 GACAAAGAAG ATGCTATCTT GAGAAAGACA GCAGCTGTTT GGGAGAGAGA  
 1251 ATTAGGGAAG CAGCACAAGG AAGACTACGA ACAAAACCAA GAAATTAGAC  
 1301 GTCTAGATAC ATCTATCTTT GAGTCTGCGG ACGTGTTCGA TGAGCAGAAA  
 1351 AAGCTTGAGA AAGATTTCCA AAGACTACAA CAAGGTPATA GCGCTCTCCA  
 1401 AGAGGAGAAA CAGOTAAAAG AAAAANTCTT AGAGAAGACT ATGATCTATT  
 1451 TTGCGGATCT TTTTGAGAGG GCTCAAAGGG AAAACATGGC CTCAGAAGAG  
 1501 AAGTTAGCGG ATTTAGAGGG TGCCGCTGCT CTTACTGAGA TCGGTAGAGA  
 1551 CGATGACTGT GTACTCACAG ATTCTGCTTC TCTCAGCCAG AAGAAGATCC  
 1601 GCGAATCTGT GGAAGAGAA TCAAGAACTCC TGAJAGACTT TGCATTTAAA  
 1651 TCTAAAGCA TGACTCAACT GOTTGCGAT GCTGTAGAGT CTCAAAAGCA  
 1701 AATCAGCRAA CTTTCAGAAC ACATAGAGA GCGAAGAAAG GATTTACGAG  
 1751 CTCTTGATPA GATGCATGCA CAAGCGATCA AAGATTGCGA AGCTGCTCAG  
 1801 AGAAATGCT GTACCTTTGA GAGGCTTCTC TCTCTGTTTC GAGAAGATGC  
 1851 TGGAAATGGA TTTGACGTAG AGGTGAGCT TCAAAGATTG CAAAGAGAAA  
 1901 ATGCACAGCT TAGAGCGGAG GTTGAAGAG TAGAGCAAGA GCAATTTCAA  
 1951 GGATTA

The PSORT algorithm predicts an inner membrane location (0.646).

The protein was expressed in *E. coli* and purified as a his-tag product and as a GST-fusion product.

The purified GST-fusion product is shown in Figure 36A. The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 36B) and for FACS analysis.

60 These experiments show that cp6879 is useful immunogen. These properties are not evident from the sequence alone.

## Example 37

The following *C.pneumoniae* protein (PID 4376767) was expressed <SEQ ID 73; cp6767>:

```

1  MIKQIGRFRF AFIFIMPLSL TSCESKIDRN RIWIVGTNAT YPPFEYVDAQ
5  51  GEVVGFDIDL AKAI SEKLGK QLEVREPAFD ALILNLKKHR IDALLAGMSI
101 TPSRQKEIAL LPYYGDEVQE LMVVSKRSL E TPVLEPLTQYS SVAVQTGTFO
151 EHYLLSQPGI CVRSFDSBLE VIMEVRYGKS PVAVLEPSVG RVVLKDFPNL
201 VATRLELPPR CWVLGCGLV AKDRPEEIQ T IQQAITDLKS EGVVLSLTKE
251 WQLSEVAYE*

```

The cp6767 nucleotide sequence <SEQ ID 74> is:

```

10 1  ATGATAAAAC AARTAGGCCG TTTTTTAGA GCATTATATT TTATAATGCC
51 51  TTTATCTTTA ACAAGTTGTG AGTCTAAAT CGATCGAAT CGCATCTGGA
101 101 TTTAGGTGAC GAATGCTACA TATCCTCCTT TTGAGTATGT GGATGCTCAG
151 151 GGGGAAGTTG TAGGTTTCGA TATAGATTTG GCAAAGCCAA TTAGTGAAAA
201 201 ACTTGGCAAG CAATTGGAAG TTAGAGAACT CGCTTTCGAT GCTTTAAATT
15 251 TAAATTAAAA AAAACATCGT ATCGATGCAA TTTTAGCAGG AATGTCACAT
301 301 ACTCCTTCGC GTCAGAAGGA AATCGCCCTG CTCCTCATT ATGGCGATGA
351 351 GGTTCAGAGG CTGATGGTGG TTTCTAAGCG GCTTTTAGAG ACCCCTGTGC
401 401 TTCCTCAATC ACAGTATTCT TCTGTGCTG TTCAGACAGG AACGTTTCAG
20 451 GAGCATATAT TTTTATCTCA GCCCGGAATT TGTGTCCGTT CTTTGTGATG
501 501 CACCTTGGAG GTGATTATGG AAGTTCGTTA TGGGAAATCT CCGGTGCGCG
551 551 TTCTAGAAC CCCTGGTAGG AAGTTCGTTA TGGGAAATCT CCGGTGCGCG
601 601 GTTCAGAAC GATTAGAGCT CCCTCCTGAA TGTGGGTGT TGGGTGTGG
651 651 TCTCGCGGTA GCTAAGATC GTCTCCTGAA AATACAAACG ATTCACAAAG
701 701 CGATTACAGA TTTAAGAGC GAAGGGGTGA TTCAATCTTT AACCAAGAAA
25 751 TGGCAACTTT CTGAAGTTGC TTACGAATAG

```

The PSORT algorithm predicts an inner membrane location (0.083).

The protein was expressed in *E.coli* and purified as a his-tag product and as a GST-fusion product. The purified his-tag product is shown in Figure 37A. The recombinant his-tag protein was used to immunise mice, whose sera were used in a Western blot (Figure 37B) and for FACS analysis (Figure 37C). The GST-fusion was also used in a Western blot (Figure 37D).

The cp6767 protein was also identified in the 2D-PAGE experiment and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6767 is a useful immunogen. These properties are not evident from the sequence alone.

## 35 Example 38

The following *C.pneumoniae* protein (PID 4376717) was expressed <SEQ ID 75; cp6717>:

```

1  MMSRLRFLRA ALGIFPILLY PMSVSAKTIV ASDKEKVGVL VYDYSVEAPQ
51 51  QILDCIDHAN FYVELCPGCT GGRTLKEVD HLEARMDLVP ELCSYIIIGP
101 101 TPTDAEDQKL LKALKBRHPN RFFYVFTGCP PSTSLAPNV IEMHKLII
40 151 DGKYCILGGT NFEEFNCPTG DEVPEKVDNP RLPVSGVRRP LAFRDQDML
201 201 RSTAFGLQLR EEFHKGQFAMV DYYAHMMFI DNPQGFAGAC PPLTLQABAE
251 251 TVFPFGDKHE DLVLVDSSKI RIVLGGPHDK QNPVPTQEYL KLIQGARSSV
301 301 KLAHMYFIPK DELLNALVDV SHNHGVHLSL ITNGCHELSP AITGPYAWGN
351 351 RINYPALLYG KRYPLWKWKF CEKLPYERV SIYEPALWET QLHKKCNMID
45 401 DEIPVIGSYN FGKKSDAFDY ESIVVIESPE VAAKANKVFN KDIGLSIPVS
451 451 HGDIFSIFYH SVHHTLGLGH LTYMPA*

```

A predicted signal peptide is highlighted.

The cp6717 nucleotide sequence <SEQ ID 76> is:

1 ATGATGAGTC GGTTCGGTTT TCQCTTGGCA GCTCTGGAA TATTTTTAT  
 51 TTGCTGCTT CTAATTTCTG TTTCAGCAAA GACATCGTA GCTTCAGACA  
 101 AGGAGAAAGT TGGAGTTCTT GTTTATGACA ATAGTGTAGA GGCCTTCAG  
 151 CAGATATTGG ATTGCATAGA TCATGCAGAA TTTTATGTAG AACTGTGTCC  
 201 CTGCAATGACA GGAGGCGAAA CGCTTAAAGA GATGTGTAGT CACTGTGAGG  
 251 CTCGTATGGA TCTGGTCCCA GAGCTCTGTA GCTATATCAT TATCCAGACC  
 301 ACGTTTACCG ATGCTGAAGA CCAAAATTTA CTCAAAGCTC TCAAAAGACG  
 351 CTATCCCAAC CGGTTTTCTT ACGTTTATAC AGGCTGCCCA CCGTCAACAA  
 401 GCATCTCTGGC TCTAATGTC ATTGAATATG ATATCAARCT TTCTATCATC  
 451 GATGGGAAAT ATTGTATTTT AGGTGGTACC AATTTTGAAG AGTTTATGTG  
 501 CACTCCAGGG GATGAGGTTC CTGAGAAAGT GATATAACCA CGTTTATTTG  
 551 TCAGTGAAGT GCTGCGGCC CTAGCATTTT GTGATACAGA TATCATGTGT  
 601 CTTTCTAGAG CATTCGGTTT CGAGCTCAGA GAAGATATPC ATAAGCAATT  
 651 TGCTATGTGG GACTACTATG CACATCATAT GTGGTTCATT GATAACTCTG  
 701 AACAGTTTGG AGGCGCTCTT CCTCCACTGA CTTTATAGACA AGCCGAGGAG  
 751 ACAGTATTTT CTGGATTGTA CAAACATGAA GATCTTTGTC TTGTGACTTC  
 801 TTCCAAGATC AGGATAGTTT TAGGTGTGCC CACGAAAG CAACCCAATC  
 851 CTGTGACTCA AGAATATTTT AAACCTATCC AGGAGCTAG ATCTTCTGTG  
 901 AAGCTTCTCT ACATGTATTT TACCTCTAAG GACGAGCTTT TAAATGCTCT  
 951 TGTGAGGCTT TCTCATATC ACGGTGTTCA TCTGAGTTTA ATTCAGAACG  
 1001 GCTGTCAATG ATTAAGTCTT GCAATACAG GACCTCATGC TTGGGGAAC  
 1051 CGTATTAACT ATTTGCGCTT GCTCTATGGG AAACGGTATC CTTTGTGAA  
 1101 AAATAGTTT TTGCGAAAAG TAAACCTTA TGAAGCGGTT TCTATTATGA  
 1151 AGTTTGTCTAT TTGCGGAACG CAGTTGACCA AGAAGTGTAT GATTATCGAT  
 1201 GATGAAATTT TTGTGATCGG AAGTTATATA TTTGGAAGA AAAGTGTGTC  
 1251 CTTTGTATAC GAAAGATATT TAGTATACGA ATCTCCAGAA GTCCGTGCAA  
 1301 AAGCTAACAA AGTCTTCAAT AAAGATATCG GATTTGTGAT TCCTGTAAAT  
 1351 CATGGCGACA TTTTCTCTTG GTATTTCAT TCCGTACACC ACATTTTGGG  
 1401 ACATTTGCGAG CTGACCTATA TGCAGCCCTA G

30 The PSORT algorithm predicts a periplasmic location (0.939).

The protein was expressed in *E. coli* and purified as a GST-fusion (Figure 38A), as a his-tagged protein, and as a GST/his fusion product. The proteins were used to immunise mice, whose sera were used in a Western blot (Figure 38B) and for FACS analysis.

35 These experiments show that cp6717 is a useful immunogen. These properties are not evident from the sequence alone.

### Example 39

The following *C. pneumoniae* protein (PID 4376577) was expressed <SEQ ID 77; cp6577>:

1 **MKKLFSTFL LVLGSTSAH** ANLGVNLKR CLEESDLGKK ETEBLEAMKQ  
 51 QFVKNAEKIE BELTSTYKIL QDEIDYMSLS DSASELRLK PEDLSGEYNA  
 101 YQSQYVQIN QSNVKRIQKL IQEVKIAAES VRSKEKLEAI LNEBAVLALA  
 151 PGTDKTTETII AILNESFKKQ N\*

A predicted signal peptide is highlighted.

The cp6577 nucleotide sequence <SEQ ID 78> is:

1 ATGAAAAAAT TATTATTTTC TACATTTCTT CTGTTTTTAG GATCAACAA  
 51 CGCAGCTCAT GCAAAATTTAG GCTATGTTAA TTTAAAGCGA TGCTTTGAAG  
 101 AATCCGATCT AGGTAAAAGG GAAATGGAAG AATTGGAAGC TATGAAACAG  
 151 CAGTTTGTAA AARATGCTGA GAAATAGAAA GRAGAACTCA CTTCTATTTA  
 201 TAAATAGTTG CAGATGGAAG ATTACATGGA AAGCTATTCG GATTTCTGCT  
 251 CTGAAGAGTT CGGAAAGAAA TTGCAAGATC TTTCAAGAGA GTACAATGCG  
 301 TACCAGTCTC AGTACTATCA ATCATCAAT CAAAGTAAT TAAACGCAAT  
 351 TCAAAAATCT ATTCAAGGAG TAAAAATAGC TGCAGATCA GTGGGCTCCA  
 401 AAGAAAAACT AGAAGCTATC CTTATTAAG AAGCTGTCTT AGCAATAGCA  
 451 CTGGGACTG ATRAAACACG CGAAATATTT GCTATTCTTA AGGAATCTTT  
 501 CAAAAACAA AACTAG

55 The PSORT algorithm predicts a periplasmic space location (0.932).

The protein was expressed in *E. coli* and purified as a his-tag product (Figure 39A) and as a GST-fusion product (Figure 39B). The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 39C) and for FACS analysis.

The cp6577 protein was also identified in the 2D-PAGE experiment.

- 5 These experiments show that cp6577 is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 40

The following *C. pneumoniae* protein (PID 4376446) was expressed <SEQ ID 79; cp6446>:

```

1  MKQPMSLIFS  SVCLGLGLGS  LSSCNQKPSW  NYHNTSTSEE  FFVHGKNSVS
51  QLPHYPSAFR  TTQIFSEHN  DPYVVAKTDE  ESRKINREIH  KNLKIKGSYI
101 PISTYGSIMH  PKSAALTILK  YRPHPIWING  YERSFNIDTG  KYLNGSRRR
151 TSHDGPKNRA  VLNLKSSGR  RCNAIGLEMT  EDEFTIARRR  EGVYSLYFVE
201 VCSYFQGNPF  VIAYLWIADE  SACSKEVLFP  KGYISLWRES  VSSSDSLNAP
251 GDSFADYLR  STFLANGTSI  LCVHESYKKV  PPQF*
```

- 15 A predicted signal peptide is highlighted.

The cp6446 nucleotide sequence <SEQ ID 80> is:

```

1  ATGAAACAGC  CCATGCTCTT  TATCTTTTCA  AGCTATGTGT  TAGGATAGG
51  TCTTGGATCT  CTTTCCTCTT  GTAATCAAAA  GCCTCTTGG  AATTACACCA
101  ACACCTCAAC  GAGCGAAGAA  TCTCTTGTTC  ATGGAACATA  GAGGTCTTCG
151  CAACCTGCCTC  ATTATCCTTC  TGCATTTCGT  ACGACTCAAA  TCTTCTCTGA
201  AGAGCACAAT  GATCCTTTATG  TCGTAGCTAA  GACTGATGAA  GAGTCTCGTA
251  AAAATTGGAG  AGAAATCCAT  AAAAATCTCA  AAATCAAAAG  TCTTACATT
301  CCCATATCGA  CTTATGGAAG  TCTGTGTCAC  CCAAATACAG  CAGCTCTTAC
351  ATTAATAACG  TATCGCCAC  ATCCATTTTG  GATAAATGSA  TACGAGCGTT
401  CTTTAAATAT  AGACACAGGA  AAGTACTTAA  AAAACGGAG  TCCGCTTAGA
451  ACTTCTCACG  ATGGTCCGAA  AAATCGAGCT  GTACTGAATC  TCATTAAATC
501  TTCCGGACGA  CGCTGTAATG  CTATAGGCTT  TGAGATGACA  GAAGAAGACT
551  TTGTAAATAG  TAGAAGCGGA  GAAGGTGTTT  ATAGCTGTGA  TCCCGTTGAA
601  GTGTGCTCGT  ACCCTCAGGG  GAATCCTTTT  GTCAATGCTT  ATGCCTGGAT
651  TGCAGATGAG  AGTGTCTGCT  CAAAAGAGGT  CCTACCTGTA  AAAGGGTACT
701  ATTCTTTAGT  CTGGGAAAGC  GTTCTCTCTT  CTGATCTCTT  GAATGCTTTT
751  GGAGATTCTT  CTGCAGAGGA  CTACCTCAGA  AGCAGGTTT  TAGCAACGGG
801  AACTTCTATA  CTCTGTGTTT  ATGAAAGCTA  TAAGAAAGGT  CCTCCTCAGC
851  CCTAA
```

- 35 The PSORT algorithm predicts an inner membrane location (0.177).

The protein was expressed in *E. coli* and purified as a his-tag product and a GST-fusion product. The GST-fusion product is shown in Figure 40A. The recombinant his-tag protein was used to immunise mice, whose sera were used in a Western blot (Figure 40B) and for FACS analysis.

- 40 These experiments show that cp6446 is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 41

The following *C. pneumoniae* protein (PID 4377108) was expressed <SEQ ID 81; cp7108>:

```

1  MSKKIKVLGH  LTLCTLFRGV  LCAAAALSNIG  YASTSQESPY  QKSIEDWKGY
51  TPTDLELLSK  EGMSEBAHVS  GNGSRIVGAS  GAGQGSVTAV  IWESHILIKHL
101  GTLGGEEASS  EGISKDGEVV  VGVSDTREGY  THAFVFDIGD  MKDLGLTGAT
151  YSVARGVSGD  GSIIVGVSA  ARGEDYGVQV  GVWKERKGIK  QKLKLPQGLW
```

201 SEANAISEDG TVIVGRGRIS RNIHIVAVKWN KNAVIVSLGTL GGVSASAEAI  
 251 SANGKIVIVGW STTNNGETHA FMHKDETMDH LGTLGGGFSEV ATGVVSADGRA  
 301 IVGFSVAVKTG EIHAFYFAEG EMEDLLTTLGG EEARVFDISS EBGNDIIGSIK  
 351 TDAGAERAYL FHHK\*

## 5 A predicted signal peptide is highlighted.

The cp7108 nucleotide sequence <SEQ ID 82> is:

1 ATGAGTAAAGA AGRTAAAGGT TCTAGGTCAT TTGAOGCTCT GCACTCTGGTT  
 51 TAGAGGAGTGT CTGTGTGCAG CGGCCCTTTC CAACATAGGA TATGCGAGTA  
 101 CTCTCTCAGGA ATCACCATAAT CAGAAGCTTA TAGAAGACTG GAAAGGTTAT  
 151 ACCTTTACAG ATCTTGAGTT ACTGAGTAAG GAAGGGTGGT CTGAAGCTCA  
 201 TGCAGTTTCT GGAAATGGCA GTAGAATTGT AGGAGCTTCG GGAGCTGGCC  
 251 AAGGTAGTGT GACTGCTGTC ATATGGGAAA GTCACTTGAT AAAACATCTC  
 301 GGCACCTTAG GTGGCAGGCC TTCACTTGCA GAGGGAATTT CAAGAGTAGG  
 351 AGAGTCTGGTC GTTGGTGGT CAGATACTAG AGAGGGATAT ACTCATGCCCT  
 15 401 TTGTCTTCGA CGGTAGAGAT ATGAAAGATC TCGGTACTCT AGGAGCTACC  
 451 TATCTCTGAP CAAGGGGTGT TTCTGAGATG GGTAGTATCA TCGTAGGAGT  
 501 CTCTGCAACT GCTCTGGGAG AGGATATACG ATGGCLAATT GGTGTCAAGT  
 551 GGGAAAAAGG GAAATCAAA CAATTGAAGT TGTTCGCTCA AGGTCTCTGG  
 601 TCTGAGCGCA ATGCAATCTC TGAGGATGGT ACGGTGATTT TCGGGAGAGG  
 20 651 GGAATCTCTCT CGCAATCACA TCGTGTCTGT AAAATGGAAT AAAATGCTGT  
 701 TGTATAGTTT GGGGACTCTC GGAGGTAGTG TCGCTTCAAG AGAGGCTATA  
 751 TCGGCAAAATG GGAAGTAAAT TGATGAGTGG TCCACGACTA ATAAATGGTA  
 801 GACTCATGCC TTATAGCACA AAGATGAGAC AATGCAAGAT CTCGGCACTC  
 851 TAGGAGGAGG TTTTCTCTGC GCAACTGGAG TTTCTGTCTA TGGGAGAGCC  
 25 901 ATCTGAGGAT TTTTCAAGCA GAAGACCGGA GAAATTCATG CTTTACTTCA  
 951 TCGCAAGGGA GAAATGGAGG ATTAAACAA CTTTGGGAGG GAAGAGAGCT  
 1001 GAGTGTTCGA CATATCTAGC GAAGGAAACG ATATCATTGG CTCTATAAAA  
 1051 ACTGACGCTG GAGCTGAACG GGCCTATCTG TTCCATATAC ATAAATAA

The PSORT algorithm predicts an outer membrane location (0.921).

30 The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 41A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 41B) and for FACS analysis (Figure 41C). A his-tagged protein was also expressed.

The cp7108 protein was also identified in the 2D-PAGE experiment.

These experiments show that cp7108 is a surface-exposed and immunoaccessible protein, and that it  
 35 is a useful immunogen. These properties are not evident from the sequence alone.

## Example 42

The following *C. pneumoniae* protein (PID 4377287) was expressed <SEQ ID 83; cp7287>:

1 **MAKKTVRSY** **RSSFHSHVIV** **AILSAGIAFE** **AHSLHSSELD** LGVFNKQFEE  
 51 HSAHVEEAQT SVLKGSDPVN PSQKSEKVL YTGVELTQGS SGESLDLADA  
 101 NLFLEHFOHLF EETTVFGIDQ KLVMSDLDTN NFSQPTQEPD TSNVASEKIS  
 151 SDTKENRKDL ETEPDSSKSG LKEVSSDLPK SPETAAVAIS EDLEISENIS  
 201 ARDPLQGLAF FYKNTSSQST SEKDSFQGT IFSGSGAMSG LGFENLAKPK  
 251 SGAAYVSDRD TVPENLVKGL SFISCSLESD GSAGVNIUV THCGDVTILD  
 301 CATGLDLLEAL RLVAKDFSRGG AVFTARNHEV QNNLAGSILS VVGNGAIVD  
 351 EKNSAEKSNQ GAPACGSFVY SNNTENTALWK ENQALSGGAI SSASDIDTGG  
 401 NCSAIEFSGN QSLIALGEHI GLTDFVGGGA LAAQTLTATR NNAVVCQVKN  
 451 TSKTHGGAIL AGTVDLNETT SEVAFKQNTA ALTGALSAN DKVLIANNFG  
 501 EILPEQNEVR NHGGAITYCG RSNPKLEQKD SGNENINI GN SGAITFLKNK  
 551 ASVLEVMTQA EDYAGGAGLW GHNVLLDSNS GNIQTIGNTG GSTFTWIGSYV  
 601 GGGAILSTDR TVTSSNSGDV VFGKNGKQCL AQKYVAPQET APVESDASST  
 651 NKDEKSLNAC SHGDHYPPKT VEEVPPSLL EEPHVVPSTD IRGGGAILAQ  
 701 HIFTDTNTGN LRFSGNLGGG EESSTVGDLA IVGGGALLST NEVNVCSNQN  
 751 VVFSDNVTSN GCDSGGAILA KKVVDISANHS VEFVCSGSKG FGGAVALNEC  
 801 SVNITNGSAA VSFSKKNRRL GGAGVAAPQG SVTICGNQGN IAPKENFVFG

|      |             |             |            |             |            |
|------|-------------|-------------|------------|-------------|------------|
| 851  | SENQRSGGGA  | LIANSSVNIQ  | DNAGDILFVS | NSTGSYGGAI  | FVGLSVASEG |
| 901  | SNPRTLTITG  | NSGDILFARN  | STQTAASLSE | KDSFGGGAIY  | TQNLKIVKNA |
| 951  | GNVSFYGNRA  | PSGAGVQIAD  | GGTVCLAEAF | GDILFEGNIN  | FDGSGFNAHL |
| 1001 | CQNDISKIVEL | SAVDQKNLIF  | QDAITYEENT | IRGLFDKDVZ  | PLSAPSLIFN |
| 1051 | SKPQDDSAQH  | KEGTIRFSRG  | VSKIPQIAAI | QEGTALASQN  | ASLWLAGLKQ |
| 1101 | ETGSSIVLSA  | GSILIRIFDSQ | VDSASAPLPE | NKEBTLVSAG  | VQINMSSPTP |
| 1151 | NKDKAVIDFPV | LADISITVDI  | LSSFVPVEQD | TLPLPPEI II | PKGTKLHNSA |
| 1201 | IDLKILDPIN  | VGYENHALLS  | SHKDILPLSL | KTAGEMGTPT  | TADASLSNKK |
| 1251 | IDVSLSPITP  | ATYGHGTGVMS | ESKMEDGRLV | VGMQPTGYKL  | NPEKGQALVL |
| 1301 | NHLWSHYTDL  | RALKQEIFAH  | HLIAQRMELD | FSTNVWSSGL  | GVVEDCQNIQ |
| 1351 | EPDFGKHHLT  | GYALGLDTPH  | VEDFLIGGCF | SQTFKTESQ   | SYKAKNDVKS |
| 1401 | YMGAAVAGIL  | AGFWLKGAF   | VYGINNDLTA | TDIGTLISIT  | GSWIGKGFIA |
| 1451 | GTSIDYRYIV  | NPRRFISAIV  | STVVPFVEAE | YVRIDLPEIS  | EQGKEVPTFQ |
| 1501 | KTRFENVAIP  | FQFALEHAYS  | RGSRAEVNSV | QLAYVDFVIR  | KGPVSLITLK |
| 1551 | DAAYSMKSYG  | VDIPCKMKA   | RLSNNTWMS  | YLSTVLAFNY  | EWREDLIAYD |
| 1601 | FNGGIRIIF*  |             |            |             |            |

A predicted signal peptide is highlighted.

The cp7287 nucleotide sequence <SEQ ID 84> is:

|      |            |             |            |            |             |
|------|------------|-------------|------------|------------|-------------|
| 1    | ATGGTAGCGA | AAAAACAGT   | ACGATCTTAT | AGGTCTTCAT | TTTCTCATTC  |
| 51   | CGTAATAGTA | GCAATATTGT  | CAGCAGGCAT | TGCTTTTGAA | GCACATTCCT  |
| 101  | TACACGAGCT | AGAACTAGAT  | TTAGGTGTAT | TCAATAACAA | GTTTGAGGAA  |
| 151  | CATTCTGCTC | ATGTTGAAGA  | GGCTCAACAA | TCGTGTTTAA | AGGGATCAGA  |
| 201  | TCTCTTAAT  | CCCTCTCAGA  | AAGAATCCGA | GAAAGTTTGT | TACACTCAAG  |
| 251  | TGCCCTCTAC | CCAGGAAGAC  | TCTGGAGAGA | GTTTGGATCT | CGCCGATGCT  |
| 301  | AATTTCCTAG | AGCATTTTCA  | GCATCTTTTT | GAAGAGACTA | CAGTATTGCG  |
| 351  | TATCGATCAA | AAGCTGGTTC  | GGTCAGATT  | AGATACAGTA | AATTTTCCCC  |
| 401  | AACCCACTCA | AGAACCTGAT  | ACAAAGTAAT | CTGTAGGTGA | GAAATCTCTC  |
| 451  | TCAGATACCA | AAGAAGATAG  | AAAAGACCTA | GAGACTGAAG | ATCCTTCAAA  |
| 501  | AAAAAGTGGC | CTTAAAGAA   | TTTCATCAGA | TCTCCCTAAA | AGTCTGAAA   |
| 551  | CTGCAGTAGC | AGCTATTTC   | GAAGATCTTG | AATCTCAGAA | AACATTTTCA  |
| 601  | GCAAGAGATC | CTCTTCAGGG  | TTTAGCATTT | TTTTATPAAA | ATACATCTTC  |
| 651  | TCAGTCTATC | TCGAAAAGG   | ATTCTTCATT | TCAGGAATAT | ATCTTTCTCG  |
| 701  | GTTCAAGAGC | TAACTCAGGG  | CTAGGTTTGT | AAAATCTTAA | GGCGCGAAAA  |
| 751  | TCGCGGCGTG | CAGTTTATTC  | TGATCGAGAT | ATTGTTTTTG | AAAATCTTGT  |
| 801  | TAAAGGATTG | AGTTTATAT   | CTTGTGAATC | TTTAGAAGAT | GGCTTCGCCG  |
| 851  | CAGGTTGAAA | CATTGTTGTG  | ACCACTTGTG | GTGATGTAAC | TCCTCACTGAT |
| 901  | TGTGCCACTG | GTTTAGACCT  | TGAAGCTTTA | CGTCTGGTTA | AAGATTTTTTC |
| 951  | TCGTGGAGGA | GCTGTTTTCA  | CTGCTCGCAA | CCATGAAGTG | CAAAATAACC  |
| 1001 | TTGCAGGTGG | AATCTATATC  | GGTTGTAGCA | ATAAAGGAGC | TATGTGTGTA  |
| 1051 | GAGAAAAATA | GTGCTGAGAA  | GTCCAATGGA | GGAGCTTTTG | CTTGCGGAAG  |
| 1101 | TTTTGTGTAC | AGTAACAAAC  | AAAACACCGC | CTTGCTGGGA | GAAATCAAG   |
| 1151 | CATTATCAGG | AGGAGCCATA  | TCTCAGCAG  | GTGATATTGA | TATTCAGGGS  |
| 1201 | AACCTGAGCG | CTATTGAATT  | TTTCAGGAAC | CAGTCTCTAA | TGTCTCTTGG  |
| 1251 | AGAGCATATA | GGGCTTACAG  | ATTTTGTAGG | TGGAGGAAGT | TTAGCTGCTC  |
| 1301 | AAGGACGCT  | TACCTTAAGA  | AATTAATGCA | TAGTCAAGAT | TGTTAAAAAC  |
| 1351 | ACTTCTAAAA | CACATGGTGG  | AGCTATTTTA | GCAGGTACTG | TTGATCTCAA  |
| 1401 | CGAACAATTT | AGCGAAGTTG  | CTTTTAAGCA | GAATACAGCA | GCTCTAACTG  |
| 1451 | GAGGTGCTTT | AAGTGCAAA   | GATTAAGGTT | TAAATTGCNA | TAACTTTTGA  |
| 1501 | GAAATCTTTT | TTGAGCAAAA  | CGAAGTGAGG | AATCACGGAG | GAGCCATTTA  |
| 1551 | TTGTGGATGT | CGATCTAATC  | CTAAGTTAGA | ACAAAAGGAT | CTTGAGAGAA  |
| 1601 | ACATCAATAT | TATTGGAAC   | TCCGGAGCTA | TCACTTTTTT | AAAAAATTAAG |
| 1651 | GCTTCTGTGT | TAGAAGTGAT  | GACACAAGCT | GAAAGATTAT | CTGTTGGAGG  |
| 1701 | CGCTTTATGG | GGGCATAATG  | TTCTTCTAGA | TTCCAATAGT | GGGAATATTTC |
| 1751 | AATTTTATAG | AAATATAGGT  | GGAAGTACCT | TCGTGAGTAG | AGATATATGTC |
| 1801 | GGTGTGGTGT | CGATTCTCTC  | TACTGATAGA | GTGACAAGAT | CTAATAACTC  |
| 1851 | TGGAGATGTT | GTTTTAAAG   | GAAACAAAGG | CCAATGTCTT | GCTCAAAAT   |
| 1901 | ATGTAGCTCC | TCAGAAACAA  | GCTCCCGTGG | AATCAGATGC | TTCACTACAA  |
| 1951 | AATAAGACGC | AGAAAGAGCCT | TAACTGCTGT | AGTCAATGGG | ATCATATATCC |
| 2001 | TCTTAAACAT | GTAGAAGAGG  | AAGTGCCACC | TTCAATGTTA | GAGAACATCT  |
| 2051 | CTGTGTGTTC | TTGCACAGAT  | ATTCTGTGTG | GTGGGGCCAT | TCAGCTCAAA  |
| 2101 | CATATCTTTA | TTACAGATAA  | TACAGGAAT  | CTGAGATTCT | CTGGGAACCT  |
| 2151 | TGGTGGTGGT | GAGAGGTCTT  | CTACTGTCCG | TGATTTAGCT | ATCGTAGGAG  |
| 2201 | GAGGTGCTTT | GCCTTCTACT  | AATGAAGTTA | ATGTTTGCAG | TAAACAAAAT  |
| 2251 | GTTGTTTTTT | CTGATAACGT  | GACTTCAAA  | GGTGTGATAT | CAGGGGAGCG  |
| 2301 | TATTTTAGCT | AAAAAAGTAG  | ATATCTCCGC | GAAACCATCG | GTTGAATTG   |

|    |      |             |             |             |             |             |
|----|------|-------------|-------------|-------------|-------------|-------------|
|    | 2351 | TCCTTAATGG  | TTCAAGGAAA  | TTCCGTGGTG  | CCGTTTGGCG  | TTTAAACGAA  |
|    | 2401 | TCAGTAACAA  | TTACGGACAA  | TGGCTCGGCA  | GTACCATCTCT | CTTAAATAG   |
|    | 2451 | AACAGCTCTT  | GGCGGTGCTG  | GAGTTGCAGC  | TCCTCAAGCG  | TCTGTAAACGA |
| 5  | 2501 | TTTGTTGGAAA | TCAGGGAAC   | ATAGCATTTA  | AAGAGACACT  | TGTTTTTGGC  |
|    | 2551 | TCGGAARACT  | AAAGATCAGG  | TGGAGGAGCT  | ATCATTTGCTA | ACTCTTCGTT  |
|    | 2601 | AAATATTACG  | GATAACCGAG  | GAGATATCCCT | ATTTGTAAGT  | AACTCTACGG  |
|    | 2651 | GATCTTATGG  | AGCTGCTATT  | TTTGTAGAT   | CTTTGGTGGC  | TTCTGAAGCG  |
|    | 2701 | AGCAACCCAC  | GAACGCTTAC  | AATTACAGGC  | AACAGTGGGG  | ATATCTTATT  |
| 10 | 2751 | TGCTAAAAAT  | AGCACGCMNA  | CAGCCGCTTC  | TTTATTCAGAA | AAAGATCTCT  |
|    | 2801 | TTGGTGGAGG  | GGCCATCTAT  | ACACAAAACC  | TCAAAATTGT  | AAAGATGCA   |
|    | 2851 | GGGAACGTTT  | CTTCTCTATGG | CACACAGAGCT | CCTAGTGGTG  | CTGGTGTCCA  |
|    | 2901 | AATTGCAGAC  | GGAGGAAGTG  | TTTGTTTAGA  | GGCTTTTGGG  | GGAGATATCT  |
|    | 2951 | TATTTGAAGG  | GAATATCAAT  | TTTGTATGGG  | GTTTCAATGC  | GATTCACTTA  |
| 15 | 3001 | TGGGGAAATG  | ACTCAAAAAT  | CGTAGAGCTT  | TCTGCTGTTT  | AGAGTAAAAA  |
|    | 3051 | TATTATTTC   | CAGATGCAA   | TTACTATGTA  | AGAGAACACA  | ATTCTGGGCT  |
|    | 3101 | TGCCAGATAA  | AGATGTCACT  | CTTTTAAAGTG | CCCTCTCAT   | AATTTTAAAC  |
|    | 3151 | TTCAAGCCAC  | AGATGTACAG  | CGCTCAACAT  | CATGAAGGGA  | CGATTACGGT  |
|    | 3201 | TTCTCGAGGG  | GTATCTAAAA  | TTCTCTAGAT  | TGCTGCTATA  | CAAGAGGGAA  |
| 20 | 3251 | CCTTAGCTTT  | ATCAAAAAAC  | GCAGAGCTTT  | GGTTGGCAGG  | ACTTAAACAG  |
|    | 3301 | GAAACAGGAA  | GTCTTATGTT  | ATTGTCGCG   | GGATCTATTCT | TCCGTATTTT  |
|    | 3351 | TGATTCCACG  | GTGTATAGCA  | GTGGCGCTCT  | TCTTACAGAA  | AATTAAGAGG  |
|    | 3401 | AGACTCTTGT  | TTCTGCCGGA  | GTTCAAATTA  | ACATGAGCTC  | TCTTACACCC  |
|    | 3451 | AATTAAGATA  | AAAGCTGTAGA | TACTCCAATG  | CTTGCAGATA  | TCATAAGTAT  |
|    | 3501 | TACTGTAGAT  | TTGTCTTCAT  | TTGTTCCTGA  | GCAAGACGGA  | ACTCTTCCTC  |
| 25 | 3551 | TTCTCTCTGA  | AATCTTCATT  | CCCTAAGGGA  | CAAAATTTACA | TTCTAATGCC  |
|    | 3601 | ATAGATCTTA  | AGATATATAGA | TCCTACCAAT  | GTGGGATATG  | AAATCATGCG  |
|    | 3651 | TTCTCTTAAGT | TCCTATAAAG  | ATATTCCATT  | AATTTCTCTT  | AAGACAGCGG  |
|    | 3701 | AAGGAATGAC  | AGGGACGCTT  | ACAGCAGATG  | CTTCTCTATC  | TAATATAAAA  |
|    | 3751 | ATAGATGTAT  | CTTACCTCTC  | GATCACACCA  | GCAACGTTATG | GTTCACACAGG |
| 30 | 3801 | AGTTTGGTCT  | GAAAGTAAAA  | TGGAAGATGG  | AAGACTTTGA  | GTGCGTTGGC  |
|    | 3851 | AACTTACGGG  | ATATAAGTTA  | AATCTCTGAG  | AGCAAGGGGC  | TCTAGTTTGT  |
|    | 3901 | AATTAATCTT  | GGAGTCAATTA | TACAGATCTT  | AGAGCTCTTA  | AGCAGGAGAT  |
|    | 3951 | CTTTGTCTCAT | CATACGATAG  | CTCAAGAAAT  | GGAGTTAGAT  | TTCTCGACAA  |
|    | 4001 | ATGTCCTGGG  | ATCAGGATTA  | GGTGTGTTG   | AAGATTGTCA  | GAACATCGGA  |
| 35 | 4051 | GAGTTTGATG  | GGTTCAACAA  | TCATCTCACA  | GGGTATGCCC  | TAGGCTTGGA  |
|    | 4101 | TACACAACTA  | GTGGAAGACT  | TCTTAATTGG  | AGGATGTTTC  | TCACAGTCTT  |
|    | 4151 | TTGTGTAATC  | TGAAGCCCAA  | TCCTACAAAG  | CTAAGAACGA  | TGTAAGAGAT  |
|    | 4201 | TATATGGGAG  | CTGCTTATGC  | GGGGATTTTA  | GCAGGTCCTT  | GGTTAATAAA  |
| 40 | 4251 | AGGAGCTTTT  | GTTTACGGTA  | ATATAACCAA  | CGATTTGACT  | ACAGATTACG  |
|    | 4301 | GTACTTTAGG  | TATTTCAACA  | GGTTCAATGA  | TAGGAAGAGG  | GTTTATCGCA  |
|    | 4351 | GGCAACAGCA  | TTGATTACCG  | CTATATTGTA  | ATCTCTACAG  | GGTTTATATC  |
|    | 4401 | GGCAATCGTA  | TCCACAGTGG  | TTCTTTTGTG  | AGAAGCCGAG  | TATGTCGGTA  |
|    | 4451 | TAGATCTTCC  | AGAATTTAGC  | GAACAGGGTA  | AAAGAGTTAG  | AACGTTCCAA  |
|    | 4501 | AAUACTCGTT  | TTGAGATGTT  | CGCCATTCCT  | TTTGGATTTC  | CTTTAGACAA  |
| 45 | 4551 | TGCTTATTCG  | CGTGCTCTAC  | GTCGTGAAGT  | GAAACAGCTA  | CAGCTTGCTT  |
|    | 4601 | ACGCTCTTGA  | TGTATATCTG  | AAGGGACCTG  | TCTCTTTGAT  | TACACTCAAG  |
|    | 4651 | GATGCTGCTT  | ATCTTGGGAA  | GAGTTATGGG  | TGAGACTTTC  | CTTGTAAAGC  |
|    | 4701 | TTGGAAGGCT  | CGCTTAGACA  | ATATAACGGA  | ATGGAATTTCA | TATTTAAGTA  |
|    | 4751 | CGTATTATAG  | GTTTAATTAT  | GAATGGAGAG  | AAGATCTGAT  | AGCTTATGAC  |
| 50 | 4801 | TTCAACGGTG  | GTATCCGCTT  | TATTTCTAG   |             |             |

The PSORT algorithm predicts an inner membrane location (0.106).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 42A.

The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 42B) and for FACS analysis (Figure 42C). A his-tagged protein was also expressed.

- 55 The cp7287 protein was also identified in the 2D-PAGE experiment and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp7287 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.



**Example 43**

The following *C.pneumoniae* protein (PID 4377105) was expressed <SEQ ID 85; cp7105>:

```

1  MSLYQKWMNS QLKXSLCYST VAALIFMIPS QESPADSLID LMLGLDPSVE
51  CLSGDGAFSV GYPTKAGSTP VEQPFKYDV SKKTFTILSV ETANQSGVAY
101 G1SYDGTITV GTCSLGAGKY NGAKWSADGT L/PLTIGITGG TSHTEARAIS
151 KDTQVIEGFS YDASGQPKAV QWASGATTVT QLADISGGSR SSVYAIISDD
201 GTIIVGSMES TITRKTITAVK WVNVPYTLG TLGGDASTGL YISDGTIVIV
251 GAANTATVTIN GNQESHAYMY KDNQMKD*

```

The cp7105 nucleotide sequence <SEQ ID 86> is:

```

10 1  GTGAGTCTAT ATCAAAATG GTGGACAGT CAGTTAAAGA AGAGCCTCTG
51  CTATTGCACT GTGCTGCTC TAATATTTAT GATTCCTTCT CAAGAATCCT
101 TTGCAGATAG TCTTATAGAT TTAATTTAG GTTTAGATCC TCGGTCGAA
151 TGCTGTGTCAG GAGATGGTCC ATTTTCTGTT GGGTATTTTA CTAAGCGGG
201 ATCGACTCCC GTAGAAATATC AGCCGTTTAA ATACAGACGA TCTAAGAAGA
251 CATTACAAAT CCTTTCGATA GAAACGGCAA ATCAGAGCGG CTATGCTTAC
301 GGAATCTCCT ACGATGGCAC GATCACGTGA GGAACGTGTA GCCTAGGTGC
351 AGGAAAATAT AACGGCGCAA AATGGAGTGC GGATGGCAGT TTAACACCT
401 TAAGTGAAT CACGGGGGGG ACCTGACATA CGGAAGCGCG TCAACCTTCT
451 AAGGATCTCT AGGTGATCGA GCGTTTCTCA TATGATGCTT CAGCGCAC
501 CAAGGCTGTG CAGTGGCGAA CGCGAGCGAC TACAGTAAAC CAATTAGCAG
551 ATATTCTCAG AGCTCTAGA AGCTCTTATG CTTATGCTAT ATCTGATGAT
601 GGCACGATTA TTGTTGGTCT TATGGAAGAC AGATTAACAA GGAAAACACT
651 AGCTGTAAAA TCGGTAAATK ATGTTCCTAC GTATCTGGGA ACCTTGGGAG
701 GAGATGCTTC TACAGTCTTT TATATTTCTG GAGACGGCAC CGTGATTGTA
751 GGTGCGGCAC ATACAGCAAC TGTAACCAAT GGAATCAGG AATCCCACGC
251 801 CTATATGTAT AAAGATAACC AATGAAAAG TTGA

```

The PSORT algorithm predicts an inner membrane location (0.100).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 43A.

The recombinant protein was used to immunise mice, whose sera were used in a Western blot

(Figure 43B) and for FACS analysis (Figure 43C). A his-tagged protein was also expressed.

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp7105 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

**Example 44**

The following *C.pneumoniae* protein (PID 4376802) was expressed <SEQ ID 87; cp6802>:

```

1  MSNQLPQCIS LGCVSYINSP PLSLQLIKRN DIRCVLAPPA DLLNLLIEGK
51  LDVALTSSLG ATSHNLGYVP GFGIAANQRI LSVNLVAAPT FPNSPQPIRA
101 ATLESRSSIG LLKVLORHLW RIPTPHILRF ITTKVLQRPV ENYDGLLLIG
40 151 DAALQHPVLV GFVTYDLASG WYDLTKLPFV FALLHSTSW KEHPLNLAM
201 BEALQQPFSS PBEVLKEAHQ HTGLPSPLLQ EYIALCQYRL GBEHYESSFEK
251 FREYYGTLYQ QARL*

```

A predicted signal peptide is highlighted.

The cp6802 nucleotide sequence <SEQ ID 88> is:

```

45 1  ATGTCTAACC AACTCCAGCC ATGTATAAGC TTAGGCTGCG TAAGTTATAT
51  TAATTCCTTT CGGTGTCCTC TACAACATCAT AAAAAAGAAC GATATTCGCT
101 GTGTCTTTCG TCCCCCTGCA GACCTCCTCA ACTTGCCTAAT CGAAGGGAAA
151 CTCGATGTTC CTCTGACCTC ATCCCTAGGA GCTATCTCTC ATAACTTGGG
201 GTATGTCCTC GGCTTTGGAA TTGCAGCAAA CCAACGTATC CTCAGTGTA

```

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251 ACCTCTATGC AGCTCCCACT TTCTTTAACT CACCGCACCC TCGGATTGCC  
 301 GCAACTTTAG AAAGTCGCTC CTCTATAGGA CTCTTAAAGC TGCTTTGTGC  
 351 TCATCTCTGG CGCATCCCAA CTCTCATAT CTTAAGATTG ATAATACAAA  
 401 AAGTACTCAG ACAACCCCTT GAAATATTATG ATGGCTCTCT CCTAATCGGA  
 451 GATGCAGCGC TACAACATCC TGTACTTCTT GGATTGTGAA CCTATGACCT  
 501 TGCCTCGGGG TGGTATGATC TTACAAAGCT ACCTTTTGTG TTTGCTCTTC  
 551 TTCTACACAG CACCTCTTGG AAGAAACATC CCTACCCCAA CCTTGGCATG  
 601 GAGGAAGCCC TCCACAGTTC CGAATCTTCA CCGGAAGAGC TCCTTAAAGA  
 651 AGCTCATCAA CATACAGGTC TGCCTCTTC TCCTTCTTCA GAATCTATG  
 701 CCCTATGCCA GTACCGTCTA GGGAAGAAGC ACTACGAAAG CTTTGAAGAA  
 751 TTCCGGGAAT ATTATGGAAC CCTCTACCAA CAAGCCGACG TGTA

The PSORT algorithm predicts an inner membrane location (0.060).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 44A.

The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 44B) and for FACS analysis (Figure 44C). A his-tagged protein was also expressed.

These experiments show that cp6802 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 45

The following *C.pneumoniae* protein (PID 4376390) was expressed <SEQ ID 89; cp6390>:

20 1 MVFSTYCMGL FFFSGAIISSC GLLVSLGVGL GLSVGLVILL LLAGLLLFKI  
 51 QSMLEREVFKA PDLLDLEDAE ERILRVKASRS LASLPKEISQ LESYIRSAAN  
 101 DNTIKTWPH KDQRLVEDVS RKLERLAAQ NYMLSELCEI SEILEEEHH  
 151 LILAQESLEW IGKSLFSTFL DMESFLNLSH LSEVRPYLAV NDPRILLEITE  
 201 ESWEVESHFI NVTSAPKKAQ ILFKNNEHSR MKKLSVQDE LLETFTYKSL  
 25 251 KRSYRELGLCL SEKMRIIHND PLFPWVDQQY QYAHAKNEFG EIAKLEEFEE  
 301 KTFPWLDEEC AISYMDCWDF LNESIQNKKS RVDRDIYSTK KIALKDRART  
 351 YAKVLEENP TTEGKIDLQD AQRAFERQSQ EPTTLEHTEIT KVRLEALQCC  
 401 FSDLREATNV RQVRFTNSEN ANDLKESFEK IDKERVRYQK EQRLYWETID  
 451 RNEQELREEI GEISRLQNRN KGYRAGYDAG RLKGLRLQWK KNLRDVEAHL  
 501 EDATMDPEHE VSKSELCSVR ARLEVLREEL MDMSPKVADI EBLLSYEERC  
 551 ILPIRENLER AYLQYKNCSE ILSKAKFFPF EDEQLVSESA NLREVGAQLK  
 601 QVQKQCPERA QKPAIFEKHI QEQKSLIKEQ VRSPDLAGVG FLKSELLSIA  
 651 ONLYIKAVVK ESIPVDVFCM QLYSYIEDN EAVVRNRLN MTERYQNFKR  
 701 SLNSIQFNGD VLLRDPVYQF EGHETRLKER ELQETTLCK KKLVAQDRLS  
 35 751 ELESRLSRR

A predicted signal peptide is highlighted.

The ep6390 nucleotide sequence <SEQ ID 90> is:

1 TTGGTATCT CATACTATTG CATGGGATTA TTTTTTTTCT CTGGAGCTAT  
 51 TCTTAGTGTG GGTCTTTTAG TGTCTCTAGG AGTGGTITTA GGACTTAGTG  
 101 TTTTAGGAGT ACTTTTACTT CTCTTAGCAG GTCTTTTGCT TTTTAAGATC  
 151 CAAAGATATG TCGAGAGGTT GCCTAAGGCT CCTGATCTAT TAGATTAGA  
 201 AGATGCAGAT GAACGGCTTA GAGTAAGGCT TAGCCGTTCT TTAGCAGGCC  
 251 TCCCGAAGGA AATCAGTCAG CTAGAGAGCT ACAATCGTTC TGCAGCTAAT  
 301 GATCTAATAA CAATTAAGAC TTGGCCGACT AAGATCAAA GACTGCTCGA  
 351 GACCGGTGTA CGAAAATTAG AGCGTCTGGC AGCTGCTCAA AACTATATGA  
 401 TTTCTGAAC TCGCGAGATT AGTGAGATTC TTGAGAGAAG GGAGCATCAT  
 451 CTAATTTTGG CTCGAGAATC TCTAGATAGG ATAGGTAAAG GTCTATTTTC  
 501 TACCTTTCTG GACATGGAAT CTTTMTTAAA TTTGAGCCAT CTATCTGAAG  
 551 TGCCTCCGTA CTAGATGTA AATGATCCTA GATTATTAGA AATTACCGAA  
 601 GAATCTTTGG AAGTAGTGAG TCATTTTCAA AATGTAAAGT CTGCTTTTAA  
 651 GAAAGCTCAG ATCTCTTTTA AGAACCAACGA ACATTTCTGG ATGAAGAAGA  
 701 AGTTAGAAAG TGTCTCAAGG TTAATGAGAA CATTTATTATA TAAGAGTTTA  
 751 AAGAGAAGTT ATCAGAAATT AGGATGCTTA AGTGAAAGA TGAGAATCAT  
 801 TCACGACAA CTCTCTTCTC CTGGGTGCA AGATCAGCAG AAGTATGCTC  
 851 ATGCTAAGAA TGAATTTGGA GAGATTGCGC GTGTTTGA GGATTTGAA  
 901 AAGACGTCTCT TCTGGTTGGA TGAGGAGTGT GCTATTTCTT ACATGAGCTG

|    |      |             |            |            |            |             |
|----|------|-------------|------------|------------|------------|-------------|
|    | 951  | TTGGGATTTT  | CTAAATGAGT | CTATTGAGAA | TAAGAACTCC | AGAOTAGATC  |
|    | 1001 | GAGATTATAT  | ATCCACGAG  | AAAATTCGAT | TAAAGGATAG | AGCCCGCACT  |
|    | 1051 | TATGCTAAGG  | TCTCTTTAGA | AGAAATCCG  | ACTCAAGAG  | GTAATAATAGA |
| 5  | 1101 | TTTGCAAGAC  | GCTCAAGAG  | CCTTGAGCG  | TCAAAGTCAG | GAGTTTATATA |
|    | 1151 | CACTGAGGCA  | TACGGAACGA | AGGTGAGAC  | TAGAGACACT | TCAACAGTCC  |
|    | 1201 | TTCTCGGATC  | TAGGCGAGGC | GACGAAGTGA | AGCCAACTTA | GGTTTACAAA  |
|    | 1251 | TTCTGAAAT   | GCAGATGAT  | TAAAGGAGAG | TTTCGAGAG  | ATAGATAAAG  |
|    | 1301 | AGCCTGTGCG  | ATATCAAAAA | GAGCAAGGC  | TCTATTGGGA | AAACAATAGAT |
|    | 1351 | CGCAATGAGC  | AAGAGCTTAC | GGAGAGATTT | GGGAGTCGC  | TTCTCTTACAA |
| 10 | 1401 | AAATCGGAGA  | AAAGGOTATA | GCGCTGGATA | TGATGCTGGG | CGTTTAAAG   |
|    | 1451 | GTTTGTTCGG  | TCAGTGGAG  | AAAAATCTCC | CGGATGCGGA | AGCCCACTTT  |
|    | 1501 | GAAAGTGCAA  | CTATGGATTT | TGAGCATGAA | GTAAGCAAGA | CGCAATTGTG  |
|    | 1551 | CAGTGTTCGG  | GCGAGGCTCG | AGGTCTTAGA | AGAAGAGCTG | ATGGATATGCT |
|    | 1601 | CTCCTAAAGT  | TCGCGATATA | GAGAGTTTGT | TGTCCTATGA | AGAGCTGCTT  |
| 15 | 1651 | ATCTTCTCTA  | TTAGGGAATA | TTTGAAGAAG | GCATACCTCC | ATATATAATA  |
|    | 1701 | GTGTTCTGAA  | ATTTTATCCA | AGGCAAGTTT | CTTCTTTCCG | GAAAGCAGAC  |
|    | 1751 | AATTGCTAGT  | TTGCGAAGCG | AATCTAAGAG | AGGTGGGTGC | CCAGTTTAAA  |
|    | 1801 | CAAGTACAGG  | GAAATATCTA | AGAGAGGCCC | CAAAAGTTCC | CAATATTGTGA |
| 20 | 1851 | AAAGCATATT  | CAGGAGCAGA | AAAGCCTTAT | TAAAGAGCAA | GTCGGAGGTT  |
|    | 1901 | TGTATCTAGC  | GCGAGTTGCG | TTTTTAAAGA | GTAGCTTTCT | TAGTATTGCT  |
|    | 1951 | TGTAACCTTT  | ATATTAAGGC | GTTTGTTAAG | GAGTCTATAC | CAGTTGATGT  |
|    | 2001 | GCTTGTATG   | CAGTTATATT | ATAGTTATTA | CGAAGATAAT | GAAAGCTGTAG |
|    | 2051 | TGCGAAACCG  | CCTTTTAAAT | ATGACGGAGA | GGTATTCAAA | TTTTTAAAGG  |
|    | 2101 | AGTTTGAATT  | CCATACAAAT | TAAATGGTAG | GTTCTTTTAC | GGGATCCGGT  |
| 25 | 2151 | CTATCAACCT  | GAAGGCTATG | AGACCAGGCT | AAAGGACCGC | GAGCTACAAAG |
|    | 2201 | AAACMACTTT  | GTCTTGTAG  | AAATTAAGAG | TGGCTCAAGA | TGTCCTTTCT  |
|    | 2251 | GAAATTAGAGT | CAGGCGTGTG | TAGGAGATAG |            |             |

The PSORT algorithm predicts a periplasmic location (0.932).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 45A.

- 30 The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 45B) and for FACS analysis (Figure 45C). A his-tagged protein was also expressed.

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

- These experiments show that cp6390 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 46

The following *C. pneumoniae* protein (PID 4376272) was expressed <SEQ ID 91; cp6272>:

|    |     |             |             |             |             |            |
|----|-----|-------------|-------------|-------------|-------------|------------|
|    | 1   | MEKRCFLPLAS | FVLMGSSADA  | LTHQEAUVKKK | NSVLSHFVKSV | SGIVTIEDXV |
|    | 51  | LNTHNLRIQ   | ANKVYVEMTV  | QQSILKIVAHQ | NVMVNYRAKT  | LYCDYLEYV  |
| 40 | 101 | DTDGCILLTNG | RFAMYFWELG  | GSKILTPET   | TVIRKGYIT   | SECFKKDLCL |
|    | 151 | SGDYLGYSSD  | SLSLGICWTL  | KVCRIFLLFL  | PPPSIMFWBI  | PKPFHFRG   |
|    | 201 | TGCGPLGSLG  | MSYSTSKAK   | FSSTFFLDSE  | FKHGYGKWFN  | LHCSQKQVE  |
|    | 251 | NVFMKSYIA   | HRLLIDMAEA  | HKRYRLHODP  | CPFHKHWNFS  | GEYHLSDSWE |
|    | 301 | TVADLFFPMF  | NKMTGQPTV   | DCVWMDNYFX  | GYLTSVSVXN  | SFQNAQELP  |
| 45 | 351 | YVTLRQNPIS  | TYNTGQVYLEX | LVSGCYLNP   | PSMIVGEMF   | SSLRLAARPK |
|    | 401 | LHKVPLPIG   | TLSSSTLGSLL | LYSDVPEIS   | SRHSQLSAKL  | QLDVRFLHK  |
|    | 451 | SYIQORHIEI  | PFVTFITETR  | PLAKNEPHY   | PSIQDAFHSI  | NLLKAGIDTS |
|    | 501 | VLSTKNTFRFP | RIHAKLWTH   | ILSNTESKPT  | FPFKTACELSL | PPGKNTVSL  |
|    | 551 | DA3YINWKHC  | WDHMYIRWEW  | IGNDNVAMTL  | ESLHRSKYSI  | LKCDRENFTL |
| 50 | 601 | DVSRPIDQLL  | DSPLSDHRNL  | ILGLFVVRPH  | PCMNIVSLRL  | YGMHRODTFN |
|    | 651 | YLEYQMILOT  | KIFEHWQLYG  | VYERREADSR  | FFFFLKLDKP  | KKPPF*     |

A predicted signal peptide is highlighted.

The cp6272 nucleotide sequence <SEQ ID 92> is:

1 ATGAAACGTT GCTTCATATT TCTAGCTTCC TTGTGTTCTTA TGGGTTCCTTC

|    |      |             |             |            |              |              |
|----|------|-------------|-------------|------------|--------------|--------------|
|    | 51   | AGCTGATGCT  | TTGACTCATC  | AAGAGGCTGT | GA AAAAGAAA  | AAC TCC TATC |
|    | 101  | TTAGTCACTT  | TAAGAGTGT   | TCTGGGATG  | TGACCATCGA   | AGATGGGGTA   |
|    | 151  | TTGAAATATC  | ATAACAACCT  | GCGGATACAA | GCCRAATAAG   | TGTATGTAGA   |
| 5  | 201  | AAATACTGTG  | GGTCAAAGCC  | TGAGAGCTGT | GCCACATGCG   | AATGTATGCG   |
|    | 251  | TGAACATAG   | GGCAAAAAC   | CTAGTTTGTG | ATTACCTTGA   | GTATACAGAA   |
|    | 301  | GATACAGACT  | CTGTCTCTCT  | TACTTAATGA | AGATTCCGCA   | TGTATCTCTG   |
|    | 351  | GTCTCTAGGG  | GCTACTTAGA  | TGACTGTAC  | CCGAGAAACC   | ATAGTCAATC   |
|    | 401  | GGAGGGGATA  | TATCTCTTAC  | TCCGAGGTC  | CCAAAAAAGA   | CCTGTCCCTC   |
|    | 451  | TCCGGAGATT  | TCTCTGGATA  | TTCCTTCAAT | AGTCTTCTTT   | CTATAGGGAA   |
| 10 | 501  | GACACATTA   | AGGGTGTGTC  | GCATTCCGAT | ACTTTTCTTA   | CCCTCAATTT   |
|    | 551  | CTATCTATCC  | TATGGAGATC  | CCTAAGCCTC | CGATAAATCT   | TCCAGAGAGA   |
|    | 601  | ACAGGAGGAT  | TTCTGGGATC  | CTATTGGGG  | ATGAGCTACT   | CGCCGATTTT   |
|    | 651  | TAGGAAGCAT  | TTCTCTCTCGA | CATTTTTCTT | GGATAGCTTT   | TTCAAGCATG   |
|    | 701  | GGCTCGGCAT  | GGGATTTCAAC | TCCCATTTGT | TCGAGAAGCA   | GGTTCCTGAG   |
| 15 | 751  | AATGCTCTCA  | ATAATGAAAAG | CTATTATGCC | CACCGCCTTC   | CTATCTGATAT  |
|    | 801  | GGCGAAGCT   | CATGATTCGCT | ATCGCTTACA | CGGAGATTTT   | TGCTTTCAAGC  |
|    | 851  | ATAAGCATGT  | AAATTTTCTT  | GGAGAAATAC | ATCTCAGCGA   | TAGTTGGGAAA  |
|    | 901  | ACTGTTGCTG  | ACATTTTCCC  | CAACAACCTC | ATGTTTGA AAA | ATACAGGCC    |
| 20 | 951  | CACACGTGTC  | GATTTGCACTT | GGAAATGACA | CTATTTTGAA   | GGGTATCTCA   |
|    | 1001 | CCTCTTCTGT  | TAAGGTAAC   | TCTTTTCAAA | ATGCCAACCA   | AGAGCTCCCT   |
|    | 1051 | TATTTAACAT  | TAAGGCACTA  | CCCGATTTCT | ATTTTATAAT   | CGGAGTGTGA   |
|    | 1101 | CCTTGA AAAC | ATCGTAGAAT  | GTGGGTATT  | AAACTTTGCT   | TTTAGCGATC   |
|    | 1151 | ATATCGTTGG  | CGAGAAATTT  | TCTTCACTAC | GTCTTGTCTG   | GGCCCTTAAG   |
| 25 | 1201 | CTCCATAAAA  | CTGTGCCCTT  | ACCTATAGGA | ACGCTCTTCT   | CCACCCGTAGG  |
|    | 1251 | GAGTCTCTCG  | ATTTACTATA  | CGGATGTTC  | TGAGATCTCC   | TGCGGCCATA   |
|    | 1301 | GTGACGTTC   | CGCGAAGCTA  | CAACTTGAT  | ATCGCTTTCT   | ATTACATAAG   |
|    | 1351 | TCTTACATTC  | AAAGACGCCA  | TATTTATAG  | CGTTCGTGTA   | CCTTCAATAC   |
|    | 1401 | AGAGACTCGT  | CCTCTAGCTA  | AGAAATGAAG | TCATTATATC   | TTTTCTATTT   |
| 30 | 1451 | AAGATGCCCT  | TCACCTCCTTA | AACTTCTGTA | AAAGCGGGAT   | AGATACCTCG   |
|    | 1501 | GTACTGAGTA  | AGACTAAACC  | TGGAATCCCG | AGAATCCATG   | CGAAGCTGTG   |
|    | 1551 | GACTACCCAC  | ATCTTGAACA  | ATACAGAAAG | CAAAACCAAG   | TTTCCCAAAA   |
|    | 1601 | CTGACATGCA  | GCTATCTCTA  | CCTTTTGGAA | AGAAAAATAC   | AGTCTCCCTTA  |
|    | 1651 | GATCGTGAAT  | GGATTTGGAA  | AAAGCACTGT | TGGGATCACA   | TGARCATAGC   |
| 35 | 1701 | TTGGGAGTGG  | ATCGGAAATG  | ACAAATGTGG | TATGACTCTA   | GAATCCCTGC   |
|    | 1751 | ATAGAAGCAA  | ATACAGCCCTG | ATTAAAGTGT | ACAGGAGAGAA  | CTTCAATTTA   |
|    | 1801 | GATGTACAGC  | GTCCCACTGA  | CCAGCTTTTA | GACTCCCCCT   | TCTCTGATCA   |
|    | 1851 | TAGGAATCTC  | ATTTTAGGGA  | AATTATTTGT | ACGCACTCAT   | CCCTGTGGGA   |
|    | 1901 | ATTACCGCTT  | ATCTCTACGC  | TATGGCTGGC | ATCGCCAGGA   | CACCTCCGAC   |
|    | 1951 | TACCTAGAAT  | ACCAGATGAT  | TCTAGGGAGC | AGATATCTCG   | AACATTTGGC   |
| 40 | 2001 | GCTCTATGGG  | GTGTATGAAC  | GCCGAGAAGC | AGATAGTCCA   | TTTTTCTTCT   |
|    | 2051 | TCTTAAAGCT  | CGACAAACCT  | AAAAAACCTC | CCTTCTAA     |              |

The PSORT algorithm predicts an outer membrane location (0.48).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 46A.

The recombinant protein was used to immunise mice, whose sera were used in a Western blot and for

45 FACS analysis (Figure 46B). A his-tagged protein was also expressed.

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6272 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

## 50 Example 47

The following *C. pneumoniae* protein (PID 4377111) was expressed <SEQ ID 93; cp7111>:

|    |     |            |            |            |             |            |
|----|-----|------------|------------|------------|-------------|------------|
|    | 1   | MFRAVIADIQ | AREILDSRGY | PTLHVKVITS | TGSGVGEARVP | SGASTGKKEA |
|    | 51  | LEPRFDSPR  | YQKGVLQAV  | KNVKEILFPL | VKGCSVYEQS  | LIDSLMMDSD |
|    | 101 | GSPNKETLGA | NAILGVSLAT | AHAAAATLRR | PLYRYLGGCF  | ACSLFPCPMN |
| 55 | 151 | LINGMHADN  | GLEPQEFMIR | PIGASSIKEA | VNMGADVFHT  | LKKLLHERGL |
|    | 201 | STGVGDEGGF | APNLASNEEA | LELLLLATER | AGPTPGDITS  | LALDCAASSF |

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251 YNVKTGTVDG RHVEEQIAIL SNLCDRYPID SIEDGLABED YDGMALLTEV
301 LGEKVQIVGD DLFVTNPELI LEGISNGLAN SVLTKPNQIG TLTEITYVIAK
351 LAQMAGYTTI ISHRSGEFTD TTIALDAVAF NAGQIKTGSLS SRSERVAKYN
401 RLMBIEEELG SEALPTDSNV PSYEDSEE*

```

5 A predicted signal peptide is highlighted.

The cp7111 nucleotide sequence <SEQ ID 94> is:

```

1 ATGTTTGAAG CTGTCATTGC CGATATCCAG GCTAGGGAAA TCTTGGATTCT
51 TCGCGGGTAT CCCACTTTAC ATGTTAAAGT AACCACTAGC ACAGGTTCTCG
101 TTGGAGAAGC TCGGGTTTCT TCAGGAGCAT CCACAGGGAA AAAAGAAGCC
151 TTAGAGTTTC GTGATACAGA TTCTCCTCGT TATCAAGGCA AAGGGGTTTT
201 GCAAGCTGTA AAAAACGTAA AAGAAATTC TTTCCCTCCT GTCAAGGGAT
251 GTAGTGTTTA TGAGCAATCC TTAATTGATT CTCTGATGAT GGATTCCTGAC
301 GGCTCTCOGA ACAAGAAGAC TCTAGGGGCC AATGCTATT TTAGGATCTC
351 TCTAGCTACA GCACATGCAG CAGCAGCAAC ACTACGCAGA CCTCTGTATC
15 401 GTTATTTAGG AGGGTGTMTT GCCTGCAGTC TTCCCTGTCC TATGATGAAT
451 CTGATCAATG GAGGCATGCA TGCAGATAAC GGCTTGGAGT TCCAAGAAAT
501 TATGATCCGT CCTATTGGAG CCTCTTCCAT CAAAGAACT GTCAACATGG
551 GTCTGACGCT TTTTCTATCT TTGAAAAAAT TACTCCAATGA AAGAGGCTTA
601 TCTACTGGAG TGGGTGACGA AGGAGGCTTC GCCCCGAATC TTGCTTCTAA
20 651 TGAAGAAGCT CTAGAGCTCC TATTTGCTGC TATTGAAAAA GCAGGCTTTA
701 CTCCAGGAAA AGATATATCG CTAGCCTTAG ACTGCGCAGC ATCTCATTC
751 TATAACGTAA AAACAGGCAC GTATGATGGG AGGCACATAT AAGAGCAAA
801 CGCAATCCTT TCTAATTAT GTGATCGCTA TCTATAGAC TCCATAGAG
851 ATGGTCTTGC TGAAGAAGAC TATGACGGGT GGGCCTTGTT AACTGAAGTT
25 901 CTTGGAGAAA AGTACAGAT TGTGGGTGAT GACCTATTGG TTACAAATCC
951 GGAATTAATA TTAGAGGGTA TTAGCAATGG ATTAGCGAAC TCTGTGTGA
1001 TTAACCAAAA CTGATAGGG ACAGCTTACTG AAACAGTGT TGTATCAAG
1051 CTTGGCGAAA TGGCTGGCTA TACTACAATT ATTCTCAATC CAGCAGGAGA
1101 AACTACGGAC ACTACGATTG CAGATCTTGC TGTGGCTTTC AACCGCGTC
30 1151 AAATCAAAAC AGGCTCTTTA TCACGTCTGT AGCGTGTTCG AAAATACAA
1201 AGACTCATGG AATTTGAAGA AGAGCTTGGG TCCGAAGCAA TTTTTCACGA
1251 TTCTAATGTA TTTTCTTAC GAGGATTCT GAGGAATAG

```

The PSORT algorithm predicts an inner membrane location (0.100).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 47A.

35 The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 47B) and for FACS analysis (Figure 47C). A his-tagged protein was also expressed.

The cp7111 protein was also identified in the 2D-PAGE experiment and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

40 These experiments show that cp7111 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 48

The following *C. pneumoniae* protein (PID 4455886) was expressed <SEQ ID 95; cp0010>:

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1 MKSQFSLVL SSTLACFTSC STVFAAATAEN IGPDSDFDGS TNGTYTPKN
51 TTTGIDYTLT GDITLQNLGD SAALTGKCFPS DTTBSLSFAG KGYLSLFLNI
45 101 KSSAEGAALS VTTDRNLSLT GFSSLTFLAA PSSVITTPBG KGNVKGCGDL
151 TFDNNGTILF KDQYCEBNGG AISTKNLSLK NSTGSIIFBG NKSSATGKKK
201 GAICATGTVD ITNNATPLF SMNIAEAAGG AINSTGNCTI TGNLSLVFSE
251 NSVTATAGNG GALSGDADVT ISGNQSVTFB GNQAVANGGA IYAKKLTAS
301 GGGVSPPLTF IIVQGTAGN GGAISILAAG ECSLSAEGAD ITFNGNAIVA
50 351 TPTPTTKRNS IDIGSTAKIT NLRAISGHSI FFYDPIPTMT AADSTDTLML
401 NKADAGNSTD YSGSIVFSGE KLSDEAKVA DNLSTLQKP VTLTGNLNV
451 KRGVTLDRKG FTQTAGSSVI MDAGTTLKAS TBEVTLGLSL IPVDSLGEKG
501 KVVIASAAS KNVALSGPIL LLDNQGNAYE NHDLGRTQDF SPVQLSALGT

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5

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551 ATTTDVPAPV TVATPPIHYGV QGTWGMTWVD DTASTPKTKT AELAWNTWGY
601 LPNPERQGPL VPNSLWGSFV DIQAIGGVIE RSALTCLCDR GFWAAGVANF
651 LDKDKKGEKR KYRHKSGGYA TCGAACTCSE NLISWAPQL FGSKDKFLVA
701 KHWDTYFAGA FYIQHITCS GFIOCLLDKL PGWSWHPKLV LEQQLAYSHV
751 SMDLKKFYTA YPEYKGSWGN NAFNMMLGAS SHSYPEVLHC FDTYAPYIKL
801 NLTYIRQDSF SRKOTEGRSF DDSNLFNLSL PTGVKPEKFS DCNDFSYDLT
851 LSYVPDLIRN DPXCTALNI SGRSMETYAN NLRARQLQVR AGSHYAFSPM
901 FEVLQGFVFE VRGSSRIYNV DLGGKQPF*

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A predicted signal peptide is highlighted.

10 The cp0010 nucleotide sequence <SEQ ID 96> is:

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1 ATGAAATCGC AATTTTCCTG GTTAGTGTCT TCCTTGACAT TGGCATGCTT
51 TACTAGTTGT TCCACTGTTT TTGCTGCAAC TGCTGAAAT ATAGGCCCTT
101 CTGATAGCTT TGACGSAAGT ACTAACACAG GCACCTATAC TCCATAAAT
151 ACGACTACTG GAATGAGACT TACTCTGACA GAGATATATA CTCTGCAAAA
201 CCTGGGGAGT TCGGCAGCTT TAACGAAGGG TTGTTTTCTT GACACTACGG
251 AATCTTTAAG CTTTGCCGGT AAGGGGTACT CACTTTCTTT TTTAAATATT
301 AAGCTTAGTG CTGAAGCGCG AGCACTTTCT GTTCAACATG ATAAAAATCT
351 GTGCTTAACA GGATTTTCGA GTCTTACTTT CTTAGCGGCC CCATCATCGG
401 TAATCACACAC CCCCTCAGGA AAGGTGCGAG TTAAATGTGG AGGGGATCTT
451 ACATTTGATA ACAATGGAACT TATTTTATTT AAACAGATT ACTGTCAGGA
501 AAATGGCGGA GCCATTTCTA CCAAGAATCT TTCTTTGAAA AACACACGG
551 GATCGATTTC TTTTGAAGGG AATAAATCGA GCGCAACAGG GAAAAAAGGT
601 GGGGCTATTT TGCTACTGTT TACTGTAGAT AITACAAATA ATACGGCTCC
651 TACCCCTCTT TCGAACAATA TTGCTGAAGC TGCAGGTGGA GCTATAAATA
25 701 GCACAGGAAA CTGTACAATT ACAGGGAATA CGTCTCTTGT ATTTTCTGAA
751 AATAGTGTGA CAGCGACCGC AGGAAATGGA GGAGCTCTTT CTGGAGATGC
801 CGATGTTACC ATATCTGGGA ATCAGAGTGT AACTTTCTCA GGAAACCAAG
851 CTGTAGCTAA TGGCGGAGCC ATTTATGCTA AGAAGCTTAC ACTGGCTTCC
901 GGGGGGGGGG GGGTATCTCC TTTTCTAACA ATAAATAGCC AAGGTACCA
30 951 TGCAGGTAAAT GGTGAGGCCA TTTCTATACT GGCAGCTGGA GAGTGTAGTC
1001 TTTACACAGA AGCAGGGGAC ATTAACCTTCA ATGGGAATGC CATTGTGCA
1051 ACTACACCCAC AAATACAAAA AAGAAATTTCT ATTGAATAGG GATCTACTGC
1101 AAAGATCACG AATTTACTGT CAAATATCTGG GCATAGCTTC TTTTCTACAG
1151 ATCCGATTAC TGCTAATACG GCTCGGGATT CTACAGATAC TTTAAATCTC
35 1201 AATAAGCTAG CTGACAGGTA TAGTACAGAT TATAGTGGGT CGATTGTGTT
1251 TTCTGGTGAA AAGCTCTCTG AAGATGAAGC AAAGTGTGCA GACAACTCA
1301 CTCTACGCTT GAAGCAGCCT GTAACCTTAA CTGACGAGAA TTTAGTACTT
1351 AAACGTGGTG TCACTCTCGA TACGAAAGGC TTTACTCAGA CCGCGGTTTC
1401 CTCTGTATTAT ATGATGCGG GCACAACTTT AAAGCAAGT ACAGAGGAGG
40 1451 TCACTTTAAC AGGTCTTTCC ATTTCTGTAG ACTCTTTAGG CGAGGCTAAG
1501 AAAGTTGTAA TTGCTGCTCT TGCACGAAGT AAAAATGTAG CCCTTAGTGG
1551 TCCGATTCTT CTTTGGGATA ACCAAGGGAA TGCTTTATGAA AATCAGCACT
1601 TAGGAAAAAAC TCAAGACTTT TCAATTGTGC AGCTCTCTGC TCTGGGTACT
45 1651 GCAACAACCTA CAGATGTTCC AGCGGTTCTT ACAGTAGCAA CTCTACGCA
1701 CTA'TGGGTAT CAAGGTACTT GGGGAATGAC TTGGGTTGAT GATACCGCAA
1751 GCACTCCAAA GACTAAGACA CGGACATTAG CTGGAGCAAA TACAGGCTAC
1801 CTCCCGAATC CTGAGCGTCA AGGACCTTTA GTTCTTAATA GCCTTTGGGG
1851 ATCTTTTTCA GACATCCAAG CGATTCAAGG TGCTATAGAG AGAAGTGCCT
50 1901 TGACTCTTTG TTCAAGTCGA GGCTCTCGGG CTGCGGGAGT CGCCAAATTC
1951 TTGATATAAG ATAAGAAAGG GGAALLACGC AATTAACCGT ATAAATCTGG
2001 TGGATATGCT ATCGGAGGTG CAGCGCAAAC TTGTTCTGAA AACTTAATTA
2051 GCTTTGCCCT TTGCGAACTT TTTGGTAGCG TGAAGAATTT CTATAGCGCT
2101 AAAATCATTA CTGATACCTA TGACGAGGCC TTCTATATCC AACACATTAC
2151 AGAATGATGT GGGTCAATAG GTTGTCTCTT AGATAAACTT CTGGCTCTTT
55 2201 GGAGTCATAA ACCCTCTGTT TTAGAAGGGC AGCTCGCTTA TAGCCACGTC
2251 AGTAATGATC TGAAGACAAA GTATACTCGT TATCTCGAGG TGAAAGGTTT
2301 TTGGGGGAAT AATGCTTTTA ACATGATGTT GGGAGCTTCT TCTCATTTCT
2351 ATCTCGAATA CCTGCATTGT TTGATATCTT ATGCTCCATA CATCAAATCT
2401 AATCTGACCT ATATACGTCA GGACAGCTTC TCGAGAGAAAG GTACAGAAAG
60 2451 AAGATCTTTT GATGACAGCA ACCCTCTCAA TTTATCTTTG CCTATAGGGG
2501 TGAAGTTTGA GAAGTTCTCT GATTGTAAATG ACTTTCTTTA TGAATCTGACT
2551 TTA'TCCTATG TTCTCGATCT TATCGCGAAT GTTCCCAATAT GCATCTACAG
2601 ACTTGTATAT AGCGGAGGCT TTGGGAAAC TTATGCCAAT AACTTAGCAC
2651 GACAGGCTCT CAAAGTGCCT GCGAGGACGT ACTACGCTTT CTCTCTATG
65 2701 TTTGAAGTGC TCGGCGAGTT TGCTTTTGAA GTTCTGAGAT CCTCAGGAGT

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2751 TTATAATGTA GATCTTGGGG GTAAGTTCCA ATTCTAG

The PSORT algorithm predicts an outer membrane location (0.922).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 48A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 48B) and for FACS analysis (Figure 48C). A his-tagged protein was also expressed.

The cp0010 protein was also identified in the 2D-PAGE experiment and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp0010 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

## 10 Example 49

The following *C.pneumoniae* protein (PID 4376296) was expressed <SEQ ID 97; cp6296>:

1 MEEVSEVYLQ VENOLESCKK RLTKMTFFAL GVRLAKKKY ESTILSDAVN  
51 RFEVLCPDIE DMLSRVEELE RMLRQABLPL LPKIALTKA FVQINSCKEK  
101 LTKVEVYFKE SPAYLFSERR LQSLNQFLQR AYKESQKVSQ LKESVRACRE  
151 QLKDVVROPE TQGVSLIKEE ILFVTSFTFR KFSYHSFRLLH VPCMRLYEYF  
201 YDDIDLERTR ARMMAMSERV RDAFQAFQEM LKEGLVREAQ ALRRETEYWL  
251 REERKSKKKH\*

The cp6296 nucleotide sequence <SEQ ID 98> is:

1 ATGGAGGAGG TGCTGAGTA TCTTCAGCRA GTAGAAAATC AGTTGGAATC  
51 CTGTTCCAAAG CGATTAAACA AGATGGAAAC TTTTGCCCTTA GGTGTGAGGT  
101 TGGAAAGCTAA AGAAGAGATA GAGTCTATCA TACTTCTCTGA TGTAGTGAAC  
151 CGTTTTCAGG TTTTATGTAG AGATATAGAA GATATGCTAT CTCGAGTCGA  
201 GGAGATAGAG CGGATGTATC GTATGGCGGA GCTTCCCTCTA CTTCCTATAA  
251 AAGAAGCGCT TACCAAGGCT TTTGTACAAC ATAACAGCTG TAAAGAGAAG  
301 TTAACCAAGG TAGAGCCTTA CTTTAAAGAG AGCCCTGCAT ATCTAACTAG  
351 TGAAGAGCGA TTGCAGAGTT TGAATCAGAC TTTACAACGT CGGTACAAG  
401 AGTCCCAAAA GGTTCAGGT TTAGAATCGG AAGTGAAGAG CTGTCGAGAG  
451 CAGCTTAAAG ATCAAGTAAG ACAGTTTGAA ACTCAAGGAG TGAGCTTGAT  
501 AAAAGAAGAG ATTCTCTTTG TGAAGTAGAT CTTTAGAAGT AAATTTAGCT  
551 ATCATTCAMT TCGATTACAT GTTCTTGTGA TGAGGTTGTG TGAGGAGTAT  
601 TATGATGACA TTGATCTAGA GAGAACTCGA GCTCGATGGA TGCGATGTC  
651 TGAGAGGATAT AGAGATGCTT TTCAGGCATT CCAGAGATG TGAAGGAAG  
701 GCCTAGTTGA AGAAGCTCAG GCTCTTAGAG AAACCGAGTA CTGTTTATAT  
751 CGAGAGGAGA GAAAGAGTAA AAGAAACAT TGA

35 The PSORT algorithm predicts a cytoplasmic location (0.523).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 49A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 49B) and for FACS analysis (Figure 49C). A his-tagged protein was also expressed.

These experiments show that cp6296 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

## Example 50

The following *C.pneumoniae* protein (PID 4376664) was expressed <SEQ ID 99; cp6664>:

1 MYLPHAQASG RNRVKADAIV LPFWHFKDAK NAASFEEFEE PSYLPALENF  
51 QOKTGEIELL YSSPKAKEER IVLLGLGKNE ELTSDVVPQT YATLTVRLERK  
101 AKCSTVNIIIL PTISLELRLA EEPLVLGSSG ILSLNYDYPR YNKVDRNLEET

-90-

151 PLSKVTVIGI VPKMADAIFR KEAAIFEGVY LTRDLVNRNA DRITPKKLAE  
 201 VALNLGKEFP SIDTKVLGKD AIAKEKMGLL LAVSKGSCVD PHFIVRVYQG  
 251 RPKSKDHTVL IGKGVTFDSG GLDLKPKGSM LTMKEDMAGG A<sup>u</sup>VLGLLSAL  
 301 AVLELPIINV GIIPATENAI DGASYKMGDV YVSGSLGSVE ICSTDABGRLL  
 351 ILADAITVAL KYCKPIRIID FATLFGAMVU SLGEVAGFV SMNDVLARDLL  
 401 LEASABTSEP LWRPLPVKRY DKTLHSDIAD MNKLGSNRAG AITAAFLQQR  
 451 FLEESSVAMA HLDIAGTAYH EKEDRYPKY ASGFGVRSIL YYLENSLSK\*

The cp6664 nucleotide sequence <SEQ ID 100> is:

1 GTGGTTTAT TTCATGCTCA AGCCTCTGGG CGTAATCGTG TTAAGGCAGA  
 10 51 TGCTATAGTC CTGCCCTTTT GGCATT<sup>u</sup>TTAA GGATGCAAAA AATSCAGCTT  
 101 CTTT<sup>u</sup>TGAGC CGAGTTTGAA CCCTCGTATC TCCCCTGCTT AGAAAACTTT  
 151 CAAGGAAAAA CCGGGGAGAT TGAACCTCTT TATAGTAGTC CTAAGGCTAA  
 201 GGAAGAAAGC ATTGTCTCTT TAGGCTTAGG GAAAAATGAA GAGCTCACCT  
 251 CTGATGTGTT TTCCAAACCC TATGCGACAC TAACCTGCTGT CTACGTA<sup>u</sup>AA  
 15 301 GC<sup>u</sup>AAAGTGT<sup>u</sup>TT CCACAGTCAA TATCATCTTA CCTACATAT<sup>u</sup> CTGATATCGG  
 351 GCTT<sup>u</sup>CTCGCC GAGAATTTCT TCTTGGGTTT GTCTCAGAGA ATTTTGTCTAT  
 401 TAAACTATGA CTACCCACGT TATAATAAGG TAGATCTGTA<sup>u</sup> TCTTGA<sup>u</sup>AACT  
 451 CCTCTTTCTA AAGTCAAGGT CATTCGGTATC GTTCCCAAAA TGGCGATGCG  
 501 TATCTTTTAGG AAGAAGCAG CATT<sup>u</sup>TTTCTA AGCGCTATAT CTCAC<sup>u</sup>CTGAGC  
 20 551 ATCTCTTGA<sup>u</sup> CAGGAATGCT GATGAATTA CCCC<sup>u</sup>TAAGAA ATTTGGCAGAG  
 601 GTTCTCTGTA ATCTGGGAAA AGAGTTCCTT AGTATTGA<sup>u</sup>TA CTAAGGTCTT  
 651 GGAAGAAAGAT CCACATCCCA AAGAGAAAT<sup>u</sup> GGGACTCTTA TTGGCTGTPT  
 701 CCAAGGTTTC TTGTGTGGAT CCACACTTTA TCGTGT<sup>u</sup>CCG TATCA<sup>u</sup>AGGTA  
 751 CGTCC<sup>u</sup>TAAGT CTAAGATCA CACCCTCTTG ATAGGGAAGG GGGTCACTTT  
 25 801 TGACTCTGGA G<sup>u</sup>TTTAGACC TCAAGCTGAG AAAT<sup>u</sup>CCATG CTTACTATGA  
 851 AAGAAGCAT GSCAGGTGG GCTACAGTCC TCGGAT<sup>u</sup>CTT C<sup>u</sup>CGGCGTTA  
 901 CAGATTTTAG AGCTTCCTAT AATGT<sup>u</sup>CACG GGGATCAT<sup>u</sup>TC CTGCTACAGA  
 951 GAATGCTATC GATGGCGCCT C<sup>u</sup>CTATAAAT GGGAGATGTC TATGTAGGAA  
 30 1001 TCTCGGGGCT TTC<sup>u</sup>GTGAGG ATTGTGA<sup>u</sup>TA CCGATGCTGA GGGACGTCTT  
 1051 ATCTCTCGCTG ATGCGAT<sup>u</sup>TAC ATATGCTTTA AATAT<sup>u</sup>TGTA AACCACAGC  
 1101 TATTTATAGAT TTGCAACTC TAACAGAGC TATGCTAGTC TCTCTAGGAG  
 1151 AAGAGGTGSC AGGT<sup>u</sup>TTCTTT TCCATAACG ATGTT<sup>u</sup>TAGC TGAAGATCIT  
 1201 TTAGAGGCGT CAGCGAAAC TCCGAGCCG TTAGGAGAC TTCTCTAGT  
 25 1251 TAAGAAGTAT GATAAAACAT TGCAATCTGA TATTGCTGAT ATGAAAAATC  
 35 1301 TAGGCAGTAA CGTGCAGGG GCTATTACAG CAGCAT<sup>u</sup>TAT<sup>u</sup> CTTCGAGAGA  
 1351 TTTT<sup>u</sup>TGGGAAG AATCTTCG<sup>u</sup>GT AGCTTGGGCA CATCT<sup>u</sup>TGATA TTGCAGGTAC  
 1401 TGCAATCAT GAAAAAGAG AAGACCGT<sup>u</sup>A TCCAAATAT GCTTCAGGTT  
 1451 TTGTTGTTCG TTTCTATTCT TATTACTTAG AATATAGTCT TTCTAGTAGT

The PSORT algorithm predicts an inner membrane location (0.268).

40 The protein was expressed in *E. coli* and purified as a GST-fusion (Figure 50A), as a his-tagged protein, and as a GST/His fusion. The proteins were used to immunise mice, whose sera were used in Western blot Western blot (50B) and FACS (50C) analyses.

The cp6664 protein was also identified in the 2D-PAGE experiment (Cpn0385) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

45 These experiments show that cp6664 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 51

The following *C. pneumoniae* protein (PID 4376696) was expressed <SEQ ID 101; cp6696>:

1 MFLIFVILIIV WCN<sup>u</sup>AFILKLC VIMGLQSR<sup>u</sup>LQ H<sup>u</sup>CEVSNQSN FDSQVKQFIY  
 50 51 ACQDKTLRQS VLKIFRYHPL LKIHDIARAV YLLMALBEGE DLGLSLFNQV  
 101 QYPSGAVBLF SCGGFPWKLK PYPAEHA<sup>u</sup>BFG LLLLLQIAE<sup>u</sup>FY EESQAVYVKM  
 151 SHFQQA<sup>u</sup>LF<sup>u</sup>FDH QGSVFLS<sup>u</sup>WMS QENSRL<sup>u</sup>LKEK TILQSGSLFQ LGMQIHPEYS  
 201 LBDPALGFWM QRTSSSAFV AASGCQSSLG AYSSGDVGVI AYGCPSGDLS  
 251 DCYYFGCCGI AKESV<sup>u</sup>CQKSH Q<sup>u</sup>TTEISFLTS TGKPH<sup>u</sup>PN<sup>u</sup>TG FSYLRDSV<sup>u</sup>YH  
 55 301 LPIRCKT<sup>u</sup>IS DQQYRVHAAL AEAT<sup>u</sup>SAMTF<sup>u</sup>S IFCKGK<sup>u</sup>ICQV VDGPR<sup>u</sup>LRSCS



351 LDSYKGPNGD DMLIGENDAI NIVSASPYME IFALQGKERF WNAFLINIP  
401 YKEEGVMLIF EKKVTSEKGR FFTKMN\*

A predicted signal peptide is highlighted.

The cp6696 nucleotide sequence <SEQ ID 102> is:

5 1 TTGACTCTAA ITTTTGTAT TATTATCGIT TGGTGCATAG CTTTTCGTAT  
51 CAATTTGTGC GTGATAATGG GGCTGCAATC CAGGTTACAA CATTTGATAG  
101 AAGTGTCCCA GAATTCGAAC TTTGATTAC AAGTAAACA GTTATCTAT  
151 GCGTGCACAG ATAAACATTT AAGGCAGTCT GTACTCAAGA TTTTCCGCTA  
201 CCATCCCTTAT CTAATAATTC ATGATATGTC TCGGGCGTC TATCTTTTGA  
10 251 TGGCTTTAGA AGAAGCGCAG GATTAGGCTT TAAGCTTTT AAATGTACAG  
301 CAGTACCCTT CAGGTGCTGT AGAACTGTTT TCTTGTGGGG GATTCCTTG  
351 GAAAGGATTA CTTTATCCTG CAGAACATGC GGAATTTGGC CTACTCCTGT  
401 TACAGATGCG AGAGTTTAT GAAGAGATC AGGCATACGT CTCTAAAATG  
451 AGTCATTTC AACAGGCAC TTTGATCAC CAAGGAGAGC TCTTCCCTC  
15 501 TCTCTGGAGC CAGGAGAACT CTGACTCCT AAAAGAAAAG ACAACTCTTA  
551 GCCAATCGTT TCTCTCCAA TTAGGAGTGC AAATTCACCC AGAATACAGT  
601 CTTGAGGATC CTGCACTAGG GTTCTGGATG CAAGAAGCG GTTCTTCATC  
651 CGCTTTTGTA GCCGCTCAG GATGTCRAAG TAGCTTGGGA GCGTATTCTT  
701 CAGGGGATGT CGGTGTTATC GCTTATGGAC CTGCTCTGAG AGACATTAGT  
20 751 GATTGTTATT ATTTTGGATG TTGTGGAAAT GCTAAAGAGT TCGTGTGCCA  
801 AAAATCTCAC CAACACTACAG AGATTCTTTT TCTCACCTCT ACAGGAAAGC  
851 CTCTACCCAG AAATACGGGA TTTTCTTACC TACGAGATCT CTATGTACAT  
901 CTGCCGATCC GCTGTAAAGT CACTATTTC GACAAGCAAT ATCCGCTGCA  
951 CGCTGCGTTG GCTGAGGCCA CCTCTGCCAT GACGTTTCTT ATTTTCTGTA  
25 1001 AGGGGAAGAA TTGTCAAGTT GTTGACGGCC CTGCTTGGG CTCTCTTCC  
1051 CTAGATTCTT ATAAAGGTCC CGGAACGAC ATTATGAMTC TTGGGGAAAA  
1101 TGACGCAATC AACATTGTTT CTGCAAGTCC CTATATGGAA ATTTTGTCT  
1151 TGCAAGGCAA AGAAAAATTT TGAAGATGAG ACTTTTGTAT TAATATTCTT  
1201 TACAAGAGAG AGGGCGTCA TTTAATTITT GAAAAAAAAG TGACCTCTGA  
30 1251 GAAAGGAAGA TTCTTTACGA AGATGAATTA A

The PSORT algorithm predicts an inner membrane location (0.463).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 51A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 51B) and for FACS analysis (Figure 51C). A his-tagged protein was also expressed.

35 This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp6696 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 52

40 The following *C. pneumoniae* protein (PID 4376790) was expressed <SEQ ID 103; cp6790>:

1 MSEHKSSKI IGIDLGTNS CVSVMEGGQA KVTSSSEGR TTPSIVAFKG  
51 NEKLVGIPAK RQAVTNPETK LGSTKRF IGR KYSEVASEIQ TTPYTIVSGS  
101 KGDVFEVDG KQVTPREITGA QILMKMKETA EAYLGSETVTE AVITVPAYFN  
151 DSQRASTKDA GRTAGLDVKK ITIPEPTAAL AYGIDKVGDK KIAVFDLGGG  
45 201 TFDISILRIG DGVFEVLSTN GDTLLGGDDF DEVLIKMMIE EFKKQEGIDL  
251 SKDNMALQRL KDAAEKAKIR LSGVSSSTEIN QPFTTMDAGD PKHIALTLTR  
301 AQPEKLAASL IERTKSPCIR ALSDAKLSAK DIDDVLLVGG MSRPMAVQET  
351 VKELFGKEPN KGVNPDEVA TGAATQGGVL GGEVKDVLIL DIVIPLSLGIE  
401 TLGGVMTTLV ERNVTIPQK QKIFSTAADN QPAVTTVLQ GERPMADNKK  
50 451 ETGRFDLTDI PPAPRGHPQT EVSFDIDANG IPHVSADKVA SKGEQKIRIE  
501 ASSGLQEDRI QRMVRDAEIN KEEDKKRREA SDAKNADSM IPRAEKATKD  
551 YKEQIPETLV KRIEERIENV RNALKDDAPI EKIKSVTEDI SKHMQIKGES  
601 MQSQSASAAA SSAANAKGGP NINTEDLKKH SPSTKPPSNN GSSEDHIEEA

651 DVEIINDDK\*

The cp6790 nucleotide sequence &lt;SEQ ID 104&gt; is:

1 ATGAGTGAAC ACAAAAATC AAGCAAAAT ATAGGTATAG ACTTAGGCAC  
 51 AACAACTCC TGCATATCTT TATGGAAAGG AGGCAAGCT AAGTAATTA  
 101 CATCATCCGA AGGAACAAGA ACCACGCCAT CGATCGTTGC CTTCAAAGGT  
 151 AATGAGAAAT TAGTGGGGAT TCCAGCAAAA CGTCAAGCAG TGACAANTCC  
 201 AGNAAAACCT CTCGGCTCTA CAAAACGGCT TATTGGCCGT AAGTACTCTG  
 251 AAGTAGCTTC GGAATTCGAA ACCGTTCTCT ATACAGTCACT CTCGGATCTC  
 301 AAAGGTGATG CCGTTTTCGA AGTGTAGTGGC AAACAATACA CTCGCCAAGA  
 10 351 AATTGGCGCA CAACACTTAA TGAATAATGAA AGAGACAGCA GAACTTTATC  
 401 TAGGCGAATC TGTACAGAAA GCATGTATCA CCGTCCCGCG ATACTTCAAT  
 451 GATTCTCAAC GAGCATCCAC AAAAGATGCT GGACGCATTG CAGGTCTAGA  
 501 TGTAACACGT ATCATTCACG AACCTACCGC AGCAGCTCTT GCCTACGGAA  
 551 TCGATAAAGT CGGTGATATA AAAATCGCTG TCTTGACCTT TGGTGGAGGA  
 15 601 ACTTTTGATA TCTCTCATCT AGAAATCGGT GATGGCGTCT TCGAAGTTCT  
 651 ATCTACAAAT GGAGATACTC TCTCGGTGG AGACGACTTT GATGAAGTCA  
 701 TTATCAAAAT GATGATCGAA GAATTCAAAA AACCAAGAGG CATTGATCTT  
 751 AGCAAGAGTA ATATGGCCCT ACAAAGACTT AAAGATGCTG CTGAGAAAGC  
 801 AAAAAATGAA CTTTCAGGAG TCTCTTCCAC AGAAATCAAT CAGCCATTCA  
 851 TCACAAATGGA TGCACAAGGA CCTAABACCC TTGCATTGAC ACTCAACAGT  
 901 GCGCAATTCG AGAAACTCGC AGGCTCTCTA ATCGAAAAGA CAAATCTTCC  
 951 ATGCATCAAA GCACCTCAGT ACACAAAAC TCCGCTTAAG GATATCGATG  
 1001 ATGTCTCTCT AGTTGGAGGT ATGTCAAGAA TGCCCGCAGT GCAAGAAACT  
 1051 GTAAAGAGAC TCTTCGCGCA AGAGCCTAAT AAAGAGTCA ACCCCGACGA  
 1101 AGTTGTGTCT ATTGGAGCCG CAATTCAAGG TGGTGTCTCT GCGCGAGAAG  
 1151 TTAAGGATGT TCTACTTCTA GACGTATACC CCTATCTCT GGGTATCGAA  
 1201 ACTCTAGGAG GCGTCATGAC GACTCTGGTA GAGAGAAATA CTCAATCCCC  
 1251 TACACAGAAA AACCAATCT TCTCCACAGC TGCTGTATAC CAGCCTGCGG  
 1301 TTACCATCGT AGTTCTCCAA GGAGAGCGTC CCATGGCCAA AGATAACAG  
 1351 GAAATCGGAA GATTGATCTC TACAGATATC CTCCGCGCTC CTCGAGGCCA  
 1401 TCTCTAAATC GAACTCTCCT TCGATATCGA TGCAAAACGA ATTTTCCATG  
 1451 TCTCAGCTAA AGATGTTGCC AGCGATAAAG AACGAAATC TCGTATCGAA  
 1501 GCAAGCTCAG GACTTCAAGA AGATGAATCG CAAAGAATGG TTCCGAGTGC  
 1551 CGAAATTAAT AAGGAAGAAG ATAAAAACG TCGTGAAGCT TCAGATGCTA  
 1601 AAAATGAAGC CGATAGCATG ATCTTCAGAG CCGAAAAAGC TATTAAGAT  
 1651 TATAAGGAGC AATTCCTCGA AACCTTTAGT AAAGAAATCG AAGAGCGAAT  
 1701 CGAAACCGTG CGCAACGCAC TCAAAAGATGA CGCTCTATAT GAAAAAATTA  
 1751 AAGAGGTTAC TGAAGACCTA AGCAAGCATA TGCAAAATAT TGGAGAGTCT  
 1801 ATSCAATGCG AGTCTCAATC AGCAGCAGCA TCATCGCGAG CCAATGCTAA  
 1851 ACGTGGACCT AACATCAATA CAGAGATTTT GAAAAAATAT AGTTTCAGTA  
 1901 CGAAGCCTCC TTCAAAATAC GGTTTCTCAG AGCAACATAT CGAAGAGGCT  
 1951 GATGTAGAAA TTATTGATA CGACGATAAG TAA

The PSORT algorithm predicts an inner membrane location (0.151).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 52A) and a his-tagged product. The proteins were used to immunise mice, whose sera were used in Western blot (Figure 52B) and FACS (Figure 52C) analyses.

The cp6790 protein was also identified in the 2D-PAGE experiment (Cpn0503).

These experiments show that cp6790 is a surface-exposed and immunooaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

## Example 53

The following *C. pneumoniae* protein (PID 4376878) was expressed <SEQ ID 105; cp6878>:

1 MNVPDSKNLH PPAYELLEIK ARITQSYKEA SAILTAIPDG ILLSETGHF  
 51 LICNSQAREI LGIDENLEIL NRSFTDVLDP TGLGFSTQE LESLKVPKTL  
 101 RLRLCKESKE KEVELFIRKN EISGYLFQI RDRSDYKQL NAIERYKNIA  
 151 ELGKMTATLA HEIRNPLSGI VGFASILKKE ISSPRHQRM LSSIIIGRSL  
 201 NNLVSMLEY TKSQPLNLKI INLQDFPSSL IPLLVSVPFH CKFVREGAP

251 LFRSIDPDRM NSVVWNLVKN AVENXNSPIT LTLHTSGDIS VTNPGTIPSE  
301 IMDKLPFPFF TTKREGNGLG LABAQKIIRL HGGDIQLKTS DSAVSFFIIL  
351 PELLALPKKE RAAS\*

The cp6878 nucleotide sequence <SEQ ID 106> is:

5 1 ATGAACGTCC CTGATTCCAA GAACCTCCAT CCTCCTGCAT ACGAACTCCT  
51 AGAGATCAAG GCTCGCATCA CACAATCTTA TAAGAAGCG AGTGCATATAC  
101 TGACAGCGAT TCTGTATGGT ATCCATATAC TTCTGTAAAC AGGACACTTT  
151 CTATATCTGA ATTCAACAAGC ACGTGAAATP CTAGGAATTG ATGAAATCTT  
201 AGAAATTCCT AATAGATCCT TTACCGATGT TCTCCCGAT ACCTGTCTTG  
251 GATTTTCTAT TCAAGAGGCT CTTGAATCTC TAAAAGTCC TAAAATCTCT  
10 301 AGACTCTCTC TCTGTAAAGA ATCTAAAGAA AAGAAGTGG AACTCTTCAT  
351 CCGTAAAAAC GAGATCAGTG GATACCTGT TATCCAAATC CGCGATCGGT  
401 CCGACTATAA ACAACTAGAA AACGCTATAG AAGATATAA AATATATCGCA  
451 GAACCTGGGA AAATGACGGC TACCCTAGCT CACGAAATC GCAATCGCT  
15 501 AAGTGGAACT GTTGGATTTG CCTCTATCCT AAGAAAGAG ATTTCTCTCT  
551 CTCGCCACCA ACGAATGCTC TCTCAATCA TCTCCGCGAC AAGGTCTCTA  
601 AATAAACCTTG TCTCTCTAT GTTAGAATAT ACAAAATCAC AACCGTTGAA  
651 CCTAAGAGAT ATAAATTTAC AAGACTTCTT CTCTCTCTCT ATCCCTCTGC  
20 701 TCTCCGCTCT TTTCCCGAAT TGCAAGTTTG TAAGAGAGGG CGCACAACCT  
751 CTATTTCAGAT CTAATAGATCC TGAATCGGATG AACAGTGTGCT TTGGAAGCT  
801 AGTGAAAAAT GCTGTAGAAA CAGGGGAATC TCCGATCACT CTGACCTCTG  
851 ATACATCGGG AGACATCTCG GTAACGAACC CCGGAACGAT TCCTTCCGAG  
901 ATCATGGACA AGCTCTTAC TCATTTCTCT ACAACAAAGA GAGAGGGAAA  
951 TGGTTAGGA CTGTGTAAGG CTCAAAAAAT TATAAGACTC CATGGAGGAG  
25 1001 ATATCCAATT AAAACAAGC GACTCCGCCG TTAGCTCTCT CATATCATC  
1051 CCCGAACCTC TAGCGGCCCT ACCCAAGAAA AGAGCCGCTA G

The PSORT algorithm predicts an inner membrane location (0.204).

The protein was expressed in *E. coli* and purified as a his-tag product (Figure 53A) and as a GST-fusion product. The recombinant GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 53B) and for FACS analysis.

These experiments show that cp6878 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 54

The following *C. pneumoniae* protein (PID 4377224) was expressed <SEQ ID 107; cp7224>:

35 1 MMKIRKIVAL AVGGSGGHIV PALSVKRAFS REGIDVLLLG KGLKNHPSLQ  
51 QGISYREIPS GLPFLVNLPIK IMSTRLSLCS GYLKARKEIK IPDPLVIGIF  
101 GSYHSLPVLV AGLSHKIPLF LHEQNLVPGK VNQLPSRYAR GIGVNSPPT  
151 KHFRCPABEV PLPKRSFSLG SFPMKRCTNH TPTICVVGGS QGAQILNCTV  
201 PQALVKLVNK YPNMYVHHIV GPKSDVMKVQ HVYNGKIVLC CVKPFEEQLL  
40 251 DVLLAADLVI SRAGATILHE ILNAKVPGIL IPYPAAGYHQ EVNAKPFYDV  
301 LBGGMILEK ELTEKLLVEK VTFALDSHNR EKQRNSLAAY SQQRSTKTFH  
351 AFICECL\*

The cp7224 nucleotide sequence <SEQ ID 108> is:

45 1 ATGATGAAGA AAATTCGAAA AGTAGCCTTG GCTGTAGGAG GTTCAGGAGG  
51 CCACATTTGTC CCAGCTCTCT CGGTAAAGGA AGCTTTTTCCT CGTAGAAGAA  
101 TAGACGTATT ACTACTAGGG AAAGGCTCTCA AGAACCATCC TTCTTTGCAA  
151 CAGGGAAATCA GCTATCGGGA AATCCCTCTCA GGACTTCCTA CAGTCTCTAA  
201 TCCCATAAAG ATCATGAGCA GACCCCTTTC TCTATGTCTA GGATACCTGA  
50 251 AAGCAAGAAA GGAACCTAAA ATTTTGTACC CTGACCTGGT CATAGGATTT  
301 GGGAGCTACC ACTCTCTTCC CGTATGTCTC GCAGACGTGT CCGATATAAT  
351 TCCCTTATTT CTACACGAAC AAAATCTAGT TCCTGAAAAA GTAAATCAAT  
401 TGTTTTCCTG CTATGCTCGA GGTATTTGAG TGAATTTCTC CCCCCTTACT  
451 AAAACACTTCC GTCGCCCGCG AGAAGAGGTC TTCTCTCTTA AACGAAGCTT  
50 501 CTCTCTTAGGA AGCCCTATGA TGAAGCGATG TACAATTCAT ACCCTTACAA  
55 551 TCTGTGTGTG TGGAGGTACT CAGGAGCAC AGATATAAA TACTTTGTGT  
601 CCCCAAGCTC TTGTCAAGCT AGTCAATAAG TACCCAATAA TGTACGTCCA

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651 TCATATTGTA GGACCTAAAA GTGATGTTAT GAAGGTGCAG CATGTTTACA  
 701 ATCGGTGAGA GGTCTCTGCG TGTTGTGAAG CGTTCGAAGA GCAACTCTTA  
 751 GATCTCTTGC TTGCGCAGAG TTGTGTCATC AGTAGGGCAG GAGCCACAA  
 801 TTTAGAAGAA ATCTCTTTGGG CAAAAGTTCC CGGAATTTPA ATTCCCTATC  
 851 CAGGAGCTTA TGGACATCAG GAAGTTAATG CTAATTTCTT TGTAGACGTC  
 901 TTAGAAGGGG GAACATATGAT CCTAGAAAAA GAATTAACAG AGAAGCTATT  
 951 AGTAGAAAAA GTACGTTTGT CTTTAGACTC CCATAACAGA GAAAAACAAC  
 1001 GCAATTCCTT AGCGGCGTAT AGTCAGCAA GGTCAACAAA AACATTCCAT  
 1051 GCATTCATTT GTGAATGCTT ATAG

10 The PSORT algorithm predicts an inner membrane location (0.164).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 54A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 54B) and for FACS analysis (Figure 54C). A his-tagged protein was also expressed.

15 This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp7224 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 55

The following *C. pneumoniae* protein (PID 4377140) was expressed <SEQ ID 109; cp7140>:

20 1 **MVRSSISFCL FFLMFLCCCT SCNRSLSLVH GLPGREENBI VLLVLSKQVA**  
 51 **AQKLFQAAAA** TAGAATBQMW DIAVPSAQIT ERLALINQAG LPRMKGTSL  
 101 DLFAKQGLVP BELQEKIRYQ EGLSBQMAST IRKMDGVVDA SVQISPTTEN  
 151 EDNLPLTASV YIKHRGVLIN PNSIMVSKIK RLIASAVFGL VPENVSUVSD  
 201 RAAYSIDITIN GPWGLTBEID YVSVMGILIA KSLTKFRLI FVVLILILFV  
 251 ISGGLMVIW KTHLIMTWG GTKGFFNPTP YTKNALBAKK AEGAAADKEK  
 301 KEDADSQGES KNAETSDEKS SDDAPEGSN EIEGA\*

A predicted signal peptide is highlighted.

The cp7140 nucleotide sequence <SEQ ID 110> is:

30 1 ATGGTTCGTC GATCTATTTC TTTTGTCTG TTCTTTCTAA TGACATTGCT  
 51 GTGCTGTACA AGCTGTAAAC GCAGGTCTCT AATTGTGCAC GGTCTTCCGT  
 101 GCAGAGAAGC GAATGAGATT GTGGTGCTTT TGGTAAGCAA AGGGGTGGCT  
 151 GCACAAAAAT TGCCTCAAGC TGCAGCGGCT ACAGCCGGAG CAGCTACTGA  
 201 GCAAAATGTGG GATATCGCGG TTTCCGTGAG ACAAAATCACA GAGGCCCTTG  
 251 CCATTCTAAA TCAAGCGGOT TCTCCACGTA TGAAAGGGAC AAGCCTGTGA  
 301 GATCTTTTITG CAAAACAAGG TCTTGTCTCT TCGAGCTTC AGGAAAAAAT  
 351 CCGTTATCAA GARGGCTTAT CAGAACAGAT GGCTCTTACG ATTAGAAAAA  
 401 TGGATGGCGT TGTGATGCC TCAGTACAGA TTTCTTTCAC TACAGAAAT  
 451 GAAGATAATC TTCTTTTAAAC AGGCTCTGTG TATATTAAAG ATCGAGGGGT  
 501 TTTGGACAAAT CCGAACAGCA TTTATGTTTC CAAAATTAAG CGCCTTATITG  
 551 CAACTGCTGT TCCAGGACTT GTGCCAGAGA ACGTCTCTGT AGTAGCGGAT  
 601 CGCGCAGCTT ATAGTGATAT TACAATTAAT GGTCTTTGGG GATTAACAGA  
 651 AGAAATCGAT TATGTTTCTG TTTGGGGTAT TATCTTTGCG AAGTCTTCGC  
 701 TCACCAAAAT CCGTCTCATC TTTTATGTCT TGATTCTCAT TTTATTTGT  
 751 ATTTCTTTGT GTCTCCTTTG GTGTCATTTG AAAACTCATA CTCTCATTAT  
 801 GACTATGGGA GGTACAAAAG GGTCTTCTCA CCCTACACCA TATACAAAGA  
 851 ATGCTTTGGA AGCCAAAGAA GCCGAGGAG CAGCTGCTGA CAAAGAGAAA  
 901 AAAGAAGATG CAGATTACCA GGGGGAAAGC AAAAAAGCGG AAACCAAGTA  
 951 TAAAGACTCT AGGTGATAAG ATGCTCCAGA AGGAAGCAAT GAAATTGAGG  
 1001 GTGCTTAG

50 The PSORT algorithm predicts an inner membrane location (0.650).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 55A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 55B) and for FACS analysis (Figure 55C). A his-tagged protein was also expressed.

These experiments show that cp7140 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 56

The following *C. pneumoniae* protein (PID 4377306) was expressed <SEQ ID 111; cp7306>:

```

1  MITQLRSLW AVLVGSSLLA LPLSGQAVGK KSRVSEKLP DVLLKEISGG
51 FSKVATKATP AVVYIESFFK SQAVTHPSPG RRGVYENFPD YFNDEFNFPF
101 FGLPSQREKF QSKRAVRGTG FLVSPDGYIV TNMHRVNDTG KIHVTLHJGQ
151 KYPATVIGLD PKTDLAVIKI KSQNLPLYLSF GNSDLHKVDG WAIATGNFFG
201 LQATVTVGVV SAKGRNQLHI ADFEFLQTFD AAINPGNSGG PLLNIDGQVI
251 GVNTAIVSGS GGYIGIGPAI PSLMANRIID QLIRDGQVTR GFLGVTLQPI
301 DAELAACYKL BKVYGALVTD VVGSGPADKA GLKQGDVIA YNGKEVDLSL
351 MFRNAVSLMN PDTRIVLKVY RBGRVIEIPV TVSQAPFKBG MSALQRVGR
401 VQNLTPETAK KLGITAPETKG LLIIISVDFGS VAASSGIPAG QLILAVNRQK
451 VSSIEDLNRT LKSDNNENIL LMVSQGDVIR FIALAPEE*
```

A predicted signal peptide is highlighted.

The cp7306 nucleotide sequence <SEQ ID 112> is:

```

1  ATGATACTA AGCAATTGCG TTCGTGGCTA GCTGTACTTG TTGGTTCAAG
51 TCTGCTAGCT CTTCCTTTAT CAGGGCAAGC TGTCGGGAAA AAGAATCTC
101 GAGUPTCCGA GCTGCCCTCA GACGTTCTTC TTAAGAGAGT CTCGGGAGGG
151 TTTTCTAAGG TCGCTACCAA GCGCACTCCC GCTGTGTGTG ACATAGAAG
201 TTTCCTCAAG AGCCAGGCTG TAACACATCC TTCTCCATGA CGCCGTGGGC
251 CTTATGAAAA TCCTTTTGAT TATTTTAATG ATGAGTTTAT CAATCGTTT
301 TTGGTCTTAC CTTCACAGAG GAAAAAACCT CAAAGTAAAG AGCGGTTTC
351 AGGAACAGGT TTCCTAGTAG CTCACGATGG CTATATTGTG ACTAATAACC
401 ATGTTGTGGA AGATACAGGT AAGATTACAG TAACTCTTCA TGATGGGCAA
451 AAGTACCCAG CAACCTGTAAT CGGACTCGAT CCTAAAAACG ACCTTGCGAGT
501 CATTAATAAT AAATCCCAAA ACCCTCCCGTA TCTTCTTTT GGAAACCTCG
551 ACCACTTAAA AGTCGGAGAT TGGGCAATTG CAATTGGAAA TCCTTCGGT
601 CTCAAGCTA CGGTACCCGT AGGTTCATC AGTGTCAAAG GAAGAATCA
651 ACTCCACATT CGAGATTTTG AAGATTTTAT TCAGACAGAT GCTCGAITTA
701 ATCCAGGCAA CTCTGGAGGC CCTCTTCTAA ATATTGATGG ACAGGTCATC
751 GGTGTTAATA CTGCCATTGT CAGTGGTAGT GGTGGCTATA TTGGAATCGG
801 GTTTCGATTT CTAAGCTTTA TGGCAATAG AATCATAGAT CAGCTGATTC
851 GTGATGGTCA AGTTACCCGA GGATTTCTAG GAGTGACTTT ACAACCTATA
901 GATCGGGAAC TCGCTGCTTG CTACAACATC GAAAGGTTT ATGCGCGTTT
951 AGTCACAGAT GTTGTTPAAG GATCTCCAGC AGATAAAGCA GGGCTAAAAAC
1001 AAGAAGATGT GATCATTTGCT TATATGGGGA AAGAAGTCGA TTCATTGAGT
1051 ATGTTCCTGA ATGCTGTTTC TTTAATGAAT CCAGATACAC GTATTGTCTT
1101 AAAGGTAGTT CGTGAAGGAA AGGTTATCGA AATACCCGTC ACAGTTTCTC
1151 AAGCTCCAAA AGAAGATGGA ATGTTGCGTT TACAGCGTGT GGGAAATCGT
1201 GTGCAAAACC TAACTCCCTGA AACTGCTAAG AAGCTGGGAA TTGCTCCAGA
1251 GACTAAAGGC ATTTTGATTA TAAGTGTGTA ACCAGGCTCT GTAGCAGCTT
1301 CTTCAGGAAT TGCTCTCTGGT CAGCTGATCC TTGCTGTGAA TAGACAAAAA
1351 GTATCTTCGA TTGAAGATCT GAATAGAACG TTAAGAGATT CTAACAATGA
1401 GAATATTCCT CTTATGGTTT CTCAAGGAGA TGTTATTCGC TTCATTGGCC
1451 TGAACCTGA AGAATTA
```

The PSORT algorithm predicts a periplasmic location (0.923).

The protein was expressed in *E. coli* and purified as a his-tag product (Figure 56A) and as a GST-fusion product (Figure 56B). The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 56C) and for FACS (Figure 56D) analyses.

The cp7306 protein was also identified in the 2D-PAGE experiment (Cpn0979) and showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

These experiments show that cp7306 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### 5 Example 57

The following *C.pneumoniae* protein (PID 4377132) was expressed <SEQ ID 113; cp7132>:

```

1 MCNSIAMKKQ KRGFVLMELL MSFTLLIALLL GTLGFWYRKI YTVQKQKERI
51 YMFYIEESRA YKQLRTLFSM SLSSSYREPG SLFSLIFDRG VYRDPKLAGA
101 VRASLIHHTK DQRLRLRCN LKQSYFETQ RLLSHVTHVV LSFQRNPDE
151 KLFPETIALTI TREPKAYPPR TLTYQFAVGK*
```

A predicted signal peptide is highlighted.

The cp7132 nucleotide sequence <SEQ ID 114> is:

```

1 ATGTGTAAC TATAGCTAT GAAAAGCAA AAGCGTGGCT TTGTGCTTAT
51 GGAATTACTC ATGTCTGTCA CTCTAATTGC TTGTATTATTA GGAACCTTAG
101 GATTTTGGTA TCGGAAATAT TATACTGTAC AAAACAAAA AGAAGCTATT
151 TATAACTTTT ATATCGAAGA AAGCGAGGCC TACAACGAGC TCAGAACCTT
201 GTTTAGCATG TCCTTGTCTT CATCTTACGA GGAGCCGCGA TCATTAATTT
251 GTTAATCTPT TGAATCGGCT GTTTATCGAG ATCTTAAGCT GCGAGGTGCG
301 GTACAGAGCT CTCCTCATCA TGACACCAAG CATCAGAGAT TCGAAGCTTCG
351 TATTTTTAAT ATTAAAGATC AGTCTTACTT TGAACACAG CGACTGCTCT
401 CCCAGCTGAC CCACTGTTGT CTTTCTCTCC AGAGAAATCC TGATCCTGAA
451 AAACTTCTGT AAACAATTCG TTTAATATA ACACGGGAAC CTAAAGCATA
501 TCCTCCAAGG ACGTTAACAT ACCAATTTCG GGTTCGGAAA TAA
```

The PSORT algorithm predicts a periplasmic location (0.915).

25 The protein was expressed in *E.coli* and purified as a his-tag product (Figure 57A) or as a GST-fusion. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 57B) and FACS (Figure 57C) analyses.

These experiments show that cp7132 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### 30 Example 58

The following *C.pneumoniae* protein (PID 4376733) was expressed <SEQ ID 115; cp6733>:

```

1 MKTSLPWWLV SSVLAFSCHL QSLANEKLLS PDDSFNGNID SGTTPPKTSA
51 TTYSLTGDVLF FYEPFGKGTPL SDSCFKQTTD NLTFPLNGHS LTFGFI DAGT
101 HAGAAASTTA NKNLTFSGFS LLSFDSFSPT TVPTGQGTLS SAGGVNLENI
151 RKLVLVAGNFS TADGGAIKGA SFLLTGTSGD ALFSNNSSST KGGALATTAG
201 ARIANTNGYV RFLSNIASTS GGAIDDEGTS ILSNNKFLYF BGNAAKTGG
251 AICNTKASGS PELIISNNKT LIPASNVLET SGGAIHAKKL ALSGGGFTEF
301 LRNVSSSATP KGGAISIDAS GELSLSAETG NITFVRNTLT TTGSTDTPWR
351 NAINIGSNK FTELRAAKNH TIFYVDPI TS EGTSSDVLKI NNGSAGALNP
401 YQGTILFSGE TLTADELKVA DNLKSSFTQP VLSGGKLLL QRGVTELETS
451 FQGBAGSLIG MDSGTTLSST AGSITITNLG INVDSGLGKQ PVSLTAKGAS
501 NKVIVSGKLN LIDIRGNIYE SHMFSDQLF SLKITTVDAD VDTNVDISL
551 IPVPAEDPNS EYFGQQQWNV NWITDTATNT KEATATWTKT GFVPSPERKS
601 ALAVCNTLWVG FTDIRSLQVL VEIGATGGEH KQGFVWSMT NPLFKGTGDN
651 RKGFRTISGG VVIGGSAHTP KDDLPTFAFC HLFARDEKDF LAHNNSTYVG
701 GTLFFKHSHL LQPNYLRLG RAKFSESAIE KFPREIPLAL DVQVFSHSD
751 NRMETHYTHSL PESEGSWSNE CIAGGIGLGL PFVLSNPHPL FKTFTPQMIV
801 EMVYVQSNSF FESSSDGRGP SIGRLNLSTL PVGAFFVQGD IGSYTTVDLS
```

851 GPFVSDVYRN NPOSTATLVH SPDSWKIRGG NLSRQAFLLR GSRNNVYNSN  
901 CELPGHYAME LRGSSRNYNV DVGTLKRF\*

A predicted signal peptide is highlighted.

The cp6733 nucleotide sequence <SEQ ID 116> is:

5 1 ATGAAGACTT CGATTCCCTTG GGTTTTAGTT TCCTCCGTGT TAGCTTTCCTC  
51 ATGTCACCTA CAGTCACTAG CTAACGAGGA ACTTTTATCA CCTGATGATA  
101 GCTTTAATGG AAAATATCGAT TCAGGAACGT TTACTCCAAA AACTTCAGCC  
151 ACAACATATT CTCATAACAGG AGATGTCTTC TTTTACGAGC CTGGAAAAGG  
201 CACTCCCTTA TCTGACAGTT GTTTTAAACA AACCCAGGAC AATCTTACCT  
10 251 TCTTGGGGAA CGGTCAATAGC TTAACGTTTG GCTTTATAGA TGCTGGCAGT  
301 CATGCAGGTG CTGCTGCATC TACAACAGCA AATAAGAAATC TTACCTTCTC  
351 AGGGTTTTCC TTACTGAGTT TTGATTCTCT TCCTAGCACA ACGGTTACTA  
401 CAGGTACAGG AACGCTTTCC TCAGCAGGAG GCGTAAATTT AGAAAATATT  
451 CGTAAACTTG TAGTTGCTGG GAATTTTCT ACTGCAGATT GTGGAGCTAT  
15 501 CAAAGGAGCG CTCTTCTCTT TAACTGGCAC TTCTGGAGAT GCTCTTTTAT  
551 GTAACAATCT TCTCATCAACA AAGGGAGGAG CAATTGCTAC TACAGCAGGC  
601 GCTCGCATAG CAATTAACAC AGGTTATGTT AGATTCTAT CTAACTATAGC  
651 GTCTACGTCA GGAGCGCGTA TCGATGATGA AGGCACGTCG ATACTATCGA  
701 ACAACAAATT TCTATATTTT GAAGGGAAATG CAGCGAAAAC TACTGGCGGT  
20 751 GCGATCTGCA ACACCAAGGC GAGTGGATCT CCGTAACCTGA TAATCTCTAA  
801 CAATAGACTC CTGATCTTTTG CTTCAAACGT AGCAGAAAACA AGCGGTGGCG  
851 CCATCCATGC TAAAAAGCTA GCCCTTTCTT CTGGAGGCTT TACAGAGTTT  
901 CTACGAAATA ATGTCTCATC AGCAACTCTT AAGGGGGGTG CTATCAGCAT  
25 951 CGATGCTCCA GGAGAGCTCA GTCTTCTCG AGAGACAGGA AACATTAOCT  
1001 TTGTAAAGAA TACCCTTACA ACACAACGGA GTACCAATAC TCTTAAACST  
1051 AATCGCATCA ACATAGGAAG TAACCGGAAA TTCAACGATC TACGGGCTGC  
1101 TAAAAATCAT ACATTTTCT TCTATGATCC CATCACTTCA GAAGGAACTC  
1151 CATCAGACGT ATTGAAGATA AATAACGGCT CTGCGGGAGC TCTCAATCCA  
1201 TATCAAGGAA CGATTCTATT TTCTGGAGAA ACCCTTAACAG CAGATGAUAT  
30 1251 TAAAGTTGCT GACAATTTAA AATCTTCAAT CACGCAACAG GTCTCCCTAT  
1301 CCGGAGGAAA GTTATTGCTA CAAAGGGAG TCACTTTAGA GAGCAGGAGC  
1351 TTCTCTCAAG AGGCGGCTTC TCTCTCGGCG ATGGATTTCAG GAACGACMTT  
1401 ATCAACTACA GCTGGGAGTA TTACAATCAC GAACCTAGGA ATCAATGTTG  
1451 ACTCCTTAGG TCTTAAGCAG CCGCTCAGCC TAAACGCAAA AGGTGCTTCA  
35 1501 AATAAAGTGA TCGTATCTGG GAAGCTCAAC CTGATTGATA TTGAAGGGAA  
1551 CATTATAGAA AGTCAATATG TCAGCCATGA CAGACTCTTC TCTCTATTAA  
1601 AAATCACGGT TGATGCTGAT GTTGATACTA ACGTTGACAT CAGCAGCCTT  
1651 ATCCCTGTTC CTGCTGAGGA TCTCAATTTCA GAATACGGAT TCCAAGGACA  
1701 ATGGAATGTT AATTGGACTA CGGATACAGC TACAAAATCA AAGAAGGCCA  
40 1751 CGCAACTTGG GACCAAAACA GGATTTTGTC CACGCCCGCA AAAAAAATCT  
1801 GCGTTAGTAT CAAATAOCTT ATGGGGAGTC TTTACTGACA TTCTCTCTCT  
1851 GCAACAGCTT GTAGAGATCG GCGCAACTGG TATGGAACAC AAACAAGGTT  
1901 TCTGGGTTC CTCCATGACG AACTTCTGCG ATAGACTCG AGATGAAAAT  
45 1951 CGCAAGGCTT TCGCTATAC CTCTGGAGGC TACGTCATCG GTGGAAGTGC  
2001 TCACATCTCT AAAGACGACC TATTTACCTT TCGGTTCTGC CATCTCTTGG  
2051 CTAGAGACAA AGATTGTTTT ATCGCTCACA ACAACTCTAG AACCTACGTT  
2101 GGAATTTTAT TCTTCAAGCA CTCTCATACC CTACAAOCCC AAACTATTTT  
2151 GAGATTAGGA AGAGCAAAAT TTTCTGAACT AGCTATAGAA AAACTCCCTA  
2201 GGGAAATTCO CTAGCCTTGG GATGTCCAAG TTTGTTTCAG CCAITCAGAC  
50 2251 AACCGTATGG AAACGCACTA TACCTCATTTG CAGAAATTCG AAGGTTCTTG  
2301 GAGCAACGAG TGTATAGCTG TGTGTTATGG CTTGAGACTT CTTTGTGTTG  
2351 TTTCCAAOCC ACATCTCTCT TCAAGACCT TCAATTCACA GATGAAAGTC  
2401 GAAATGGTTT ATGTATCAACA AAATAGCTTC TTGAAAGGCT CTAGTGATGG  
55 2451 CCGTGGTTTT AGTATTTGGA GGCTGCTTTA CTTCTGATTT CTAATCTCTA  
2501 CGAAATTCGT CGAGGGGAGT ATCGGAGATT CTTCACTCTA TGATCTCTCA  
2551 GGATTTCTTG TTTCCGATGT CTATCTTAAC AATCCOAAAT CTACAGCGAC  
2601 TCTTGTGATG AGCCAGACT CTGGAAGAAAT TCGCGGTGTC AATCTTTCAA  
2651 GACAGGCAAT TTACTAGAG GGTGACAAACA ACTACGCTCA CAACTCCAAAT  
70 2701 TGTGAGCTCT TCGGACAATTA CGCTATGGAA CTCGTGGGAT CTTCAAGGAA  
2751 CTAATATGTA GATGTTGTA CCAAACTCG ATTTCTAG

The PSORT algorithm predicts an outer membrane location (0.924).

The protein was expressed in *E.coli* and purified as a his-tag product, as shown in Figure 58A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 58B) and for FACS (Figure 58C) analyses. A GST-fusion protein was also expressed.

The cp6733 protein was also identified in the 2D-PAGE experiment (Cpn0451).

- 5 These experiments show that cp6733 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 59

The following *C.pneumoniae* protein (PID 4376814) was expressed <SEQ ID 117; cp6814>:

10

```

1 MHDALLSILA IQELDIRMIR LMRVKEHKQ ELAKVQSLS DIRRKVQRIE
51 LEMENLKQTI RDGENRIQEI SEQINKLENQ QAAVKRMDEF NALTQEMTTA
101 NKERRSLEHQ LSDLMRDQAG GEDLIVSLKE SLASTENSSS VIEKIEFISI
151 KKINEBKAL LEQRTELKHA TNPELLSIYE RLLNKKRQV VPIENRVCS
201 GCHIVLTPQH ENLVRKKDRL TFCHECSRIL YWQESQVNAQ ENSTAKRRRR
251 RAAV*
```

- 15 The cp6814 nucleotide sequence <SEQ ID 118> is:

```

1 ATGCATGACG CACTTCTAAG CATTTGGCT ATTCAAGAGC TTGATATTAA
51 AATGATTCGC CTTATGCGCG TAAAGAAAGA ACATCAGAAA GAATTGGCTA
101 AAGTCCAATC TTATAAAGT GATATTCGTA GAAAAGTCA GGAAGAAGAA
151 CTCGAAATGG AGAATTTGAA AACTCAAAAT CGAGATGGAG AGAATCGCAT
201 CCAAGAGATT TCTGAACAAA TCAATAAATT AGAATTCAG CAAGTGCATG
251 TAAAAAATAT GGATGAGTTT AACGCTCTTA CCCAAGAAAT GACTACAGCA
301 AACAAAGAAC GTGCTCTTT AGAGCACCAG CTIAGCGATC TCAATGGATAA
351 GCAAGCTGGA GCGAAGACC TTATTGTCTC TCTAAAGAA AGCTTAGCTT
401 CTACAGAAAA TAGTAGCAGT GTCATTGAAA AAGAAATTTT TGAAAGCATC
451 AAAAAGATTA ATGAAGAAGG CAAAGCTTTG CTTGAACAA GGCACAGATT
501 AAAGCATGCG ACGAATCCCG AACTACTCAG CATCTATGAG CGTCTATTAA
551 ACAATAAAAA AGATCGCGTT GTTGTTCCTA TTGAAAATCG TGCTCGAGT
601 GGTGTTCATA TTGTCTAAC TCTCTAACAC GAAATCTTG TAAGAAGGAA
651 AGACCGACTC ATTTTTCGCG AACATTGCTC TCGAATCTCT TATTGGCAAG
701 AATCCCAAGT CAATGCTCAG GAAATTCAC CAGCAAACG TGTCTGCTGT
751 CGCGCAGCTG TATAA
```

The PSORT algorithm predicts an inner membrane location (0.070).

The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 59A) or his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used in Western blot (Figure 59B) and FACS (Figure 59C) analyses.

These experiments show that cp6814 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 60

The following *C.pneumoniae* protein (PID 4376830) was expressed <SEQ ID 119; cp6830>:

40

```

1 MKWLPAFAVF AAVLPALFAP QDPASVEIST SHTGSSDPTS DAALTGFTQS
51 STEIDGTTYT IVGDITFSTP TNIPVPVVTPT DANDSSSSNS KGGSSSSGAT
101 SLIRSSNLHS DTFPKDSVL DLYHLFPFSA SNLNPALLS SSSSSGSSSS
151 SSSSSSGSAS AVVAADPRGG AAFYSNEANG TLTPPTDSGN FGSLLTLNKL
201 MFGDGAATYS KGFIVPTSLK NLTPQTQNESQ KSGGAAYTBG ALITQALVEA
45 251 VFTPTMTSAG KGGAIYVKEA TLFNALDSLK FEKNTSQAG GGIITESTLT
301 ISNITKSTFP ISNKASVFAF APEPTSPFAS SLINSTFIDT STLQTRASA
351 TPAVAVAAV TPTFISTQET AGKGGAIYAK QGISISTFID LTFKSSNSAV
```



|    |      |             |             |             |             |             |
|----|------|-------------|-------------|-------------|-------------|-------------|
|    | 401  | DATLTVDSST  | IGESGGAIFA  | ADSIQIQQCT  | GTTLFSGQNTA | NKSGGGIYAV  |
|    | 451  | GQVTLIEDIAN | LQMTNNNTCKG | EGGAIYTKKA  | LTINNAGAILT | TFSGNTSTDN  |
|    | 501  | GGAIAPAVGGI | LDLSLVEVRF  | SKNKTGNYS A | PTTKAASNTA  | PVVSSTSTAA  |
| 5  | 551  | SPAVAAAAA   | PVTNAAGGGA  | LYSTEGTLVS  | GITSILSFBN  | NCCQNGGGGA  |
|    | 601  | YVTKTFQCSD  | SHRLQPTSNK  | AADGGGLYC   | GDDVLTNLNLT | GKTLFQENSS  |
|    | 651  | EKHGGGLSLA  | SGKSLMTSL   | ESFLCNANTA  | KENGGGANVP  | ENIVLTFTYT  |
|    | 701  | PTPNEPAPVQ  | QPVYGEALVT  | GNTATKSGGG  | IYTKNAAFSN  | LSVSTFDQNT  |
|    | 751  | SSENGALLT   | QKAAADTDCS  | PTTITNVNIT  | NNIATGNGGG  | IAGGKAHFDR  |
| 10 | 801  | IDNLTVQSNQ  | AKKGGGVYLE  | DALILEKVT   | GSVSGNTATE  | SGGGIYAKDI  |
|    | 851  | QLQALPGSFT  | TTDNKVETSL  | TTSTNLVGGG  | IYSSGAVTIT  | INSGTFTGIT  |
|    | 901  | NSVINTATSQ  | DADIQGGGIY  | ATPSLSINQC  | NTPILFSNNS  | AATKKTSTTK  |
|    | 951  | QIAGGAIFSA  | AVTIENNSQF  | IIFLANSKAS  | EATTAATAGN  | KDCSCGGAIAA |
|    | 1001 | NSVLTNNNPE  | TFKGNYAET   | GGAIGCLDLT  | NGSPPRKVS I | ADNGSVLFDQ  |
| 15 | 1051 | NSALNRGGAI  | YGETIDISRT  | GATFIQNSNK  | HGSAICCSBT  | ADTLAPNSQL  |
|    | 1101 | IFENNRVTET  | TATTKASINN  | LGAALFYGNB  | TSDVITSLSA  | ENGSIFFKNK  |
|    | 1151 | LCTATNKYCS  | IAGNVKPTAI  | EASAGKAI SF | YDAVNVS TRE | TNAQEKLINE  |
|    | 1201 | KATSTGTLLF  | SGELHKNKSY  | IPQKVTFAHG  | NLLCGKABL   | SVVSFTQSPG  |
|    | 1251 | TTITMGPSGV  | LSNHKKKAGG  | IANNVLIDF   | SELVPTKML   | TVAPPTLKLIV |
| 20 | 1301 | SRTWADSKDX  | IDTGTGVTLI  | DPKGLVQMS   | YLQEDRDYPL  | PHINDSAGSA  |
|    | 1351 | VTATVTYTLQ  | NLGAKKYGLG  | TPMLDPNSSG  | SKILLKWTDF  | KYLWSPYIFR  |
|    | 1401 | DNHFYINELW  | GAQNSLVTVK  | QGLTLNMLAN  | ARFEDPAPIN  | PWASALIGSPL |
|    | 1451 | REKRVSNRDS  | FTYHGRGYTA  | AVDAKPRQEF  | TLGAASFQVE  | GHAESYHLDD  |
|    | 1501 | NVHKHKGSGHS | TQASLYAGNI  | PYPFAIRSRP  | ILFQCVAITYG | YMQHDDTTTYQ |
| 25 | 1551 | PSIEEKNMAN  | NDSIAWFLDF  | RFSVDLKEPO  | PHSTARLTPTV | TAEBYTRIRQ  |
|    | 1601 | EKPTELDYPD  | RSPSACSYGN  | LAIPGFSVD   | GALANREIIL  | YIKVYSAAIPL |
|    | 1651 | VILRNINPKAT | YEVLTSTKEK  | NVNVLPTRN   | ARAEEVSSQI  | YLGSYWTLYG  |
|    | 1701 | TYTIDASMMT  | LVQMGANGIR  | PVP*        |             |             |

A predicted signal peptide is highlighted.

The cp6830 nucleotide sequence <SEQ ID 120> is:

|    |      |             |             |            |            |             |
|----|------|-------------|-------------|------------|------------|-------------|
| 30 | 1    | ATGAAGTGGC  | TACCAAGCTAC | AGCTGTTTTT | GCTGCCGTAC | TCCCCGCACT  |
|    | 51   | AACAGCCTTC  | GGAGATCCCG  | CGCTGTTTGA | AATAAGTACC | AGCCATACAG  |
|    | 101  | GATCCGGGGA  | TCTCATCAAG  | GACGCTGCTT | TAAACAGGAT | TACACAAAGT  |
|    | 151  | TCCACAGAAA  | CTGACGGTAT  | TACCTATACC | ATTGTCGGTG | ATATCACTCT  |
|    | 201  | CTCTACTTTT  | ACGAATATTC  | CTGTCCCGCT | AGTAACTCCA | GACGCCAACG  |
| 35 | 251  | ATAGTTCGAC  | CAATAGCTCT  | AAAGGAGGAA | GTAGCAGTAG | TGGAGCTACA  |
|    | 301  | TCTCTAATCC  | GATCCCTCAA  | CTTACACTCC | GATTTTGATT | TTACAAAAGA  |
|    | 351  | TAGCGTGTGA  | GACCTCTATC  | ACCTTTTCTT | TCCTTCAGCT | TCAAATACTC  |
|    | 401  | TCAATCCTGC  | ACTCCTTTCT  | TCCAGTAGCA | CGGTTGGATG | CTCGAGCAGC  |
|    | 451  | AGTAGCTCCT  | CATCATCTGG  | AAGTGCATCT | GCTGTGTGTG | CTCGGACCCC  |
| 40 | 501  | AAAAGGAGGC  | GCTGCCCTTT  | ATAGTAACGA | GGCTAACGGA | ACTTTAACTC  |
|    | 551  | TCACCTACAGA | CTCTGGAAT   | CCCGGCTCCC | TGACTCTTCA | GAATCTTAAA  |
|    | 601  | ATGACCGGAG  | ATGGAGCCGC  | CATCTACTCG | AAAGGTCCTC | TAGTATTATC  |
|    | 651  | TGGTTTAAAA  | AATCTAACTC  | TTACAGGAAA | TGAATCTCAG | AAATCTGGAG  |
|    | 701  | GTGCTGCCTA  | TACTGAAGGC  | GCATCACAA  | CACAAGCAAT | CGTTGAAGCC  |
| 45 | 751  | GTAACCTTTA  | CTGGCAACAC  | CTCGCAGGGG | CAAGGAGGCG | CTATCTATGT  |
|    | 801  | TAAAGAGACT  | ACCCATATCA  | ATGCTCTAGA | CAGCTCTCAA | TTTGAAAAAA  |
|    | 851  | ACACTTCTGG  | GCAAGCTGGT  | GGTGGNATCT | ATACAGAGTC | TACGCTCACA  |
|    | 901  | ATCTCGAACA  | TCACAAAATC  | TATTGAAATT | ATCTCTAATA | AAGCTTCTGT  |
|    | 951  | CCCTGCCCCC  | GCTCCTGAGC  | CCACTCTCCC | GGCTCCAAGT | AGCTTAAATA  |
| 50 | 1001 | ATTCTACAC   | GATCGATACC  | TGCACTCTCC | AAACCGGAGC | AGCATCCGCA  |
|    | 1051 | ACTCCAGCAG  | TGGCTCTCTG  | TGCTGCCGTA | ACTCCAACAC | CAATCTCTAC  |
|    | 1101 | TCAAGAGACC  | CGAGGAAATG  | GAGGCGCTAT | CTATGCTAAA | CABGGTATTT  |
|    | 1151 | CGATATCCAC  | GTTTAAAGAT  | CTGACCTTCA | AGTCTAACTC | TGCACTCGTA  |
| 55 | 1201 | GATGCCACCC  | TTACTGTCTG  | TCTGACACT  | ATTGGAGAAT | CTGGAGGTGC  |
|    | 1251 | TATCTTTGCA  | CGAGACTCTA  | TACAAATCCA | ACAAGTCACG | GGAAACCACT  |
|    | 1301 | TATTCAGTGG  | CAATACTGCC  | AATAAGTCTG | TGGGGGGTAT | TTACGCTGTA  |
|    | 1351 | GGACAAGTCA  | CCCTAGAAGA  | TATAGCGAAT | CTGAAGATGA | CCBACAACAC  |
|    | 1401 | CTGTAAAGGT  | GAAAGTGGAG  | CCATCTACAC | TAAAAGGCTT | TTAACATATCA |
|    | 1451 | ACAAAGGTCG  | CATCTCACT   | ACATTTTCTG | GAAATCAATC | CACAGATAAT  |
| 60 | 1501 | GGTGGGCTA   | TTTTTGCTGT  | AGGTGGCATC | ACTCTCTCTG | ATCTTGTAGA  |
|    | 1551 | AGTCGCGTTT  | AGTAAAAATA  | AGACCGGAAA | TTATTTCCGT | CCTATTACCA  |
|    | 1601 | AAGCGGCTAG  | CAACACAGCT  | CTGTAGTTT  | CTAGCTCTAC | AACTGCTGCA  |
|    | 1651 | TCTCTGCGG   | TCCCTGCTCG  | CGCTGCAGCA | CCTGTATCAA | ACGCAACAAA  |
|    | 1701 | AGGAGGGGCT  | TTATATAGTA  | CAGAAGGACT | GACTGTATCT | GGATACATAT  |
| 65 | 1751 | CGATATTGTC  | GTTTGAAGAC  | AACGAATGCC | AGAATCAAGG | AGGTGGGGCT  |

-100-

|      |             |             |             |             |            |
|------|-------------|-------------|-------------|-------------|------------|
| 1801 | TAGGTTACTA  | AAACCTTCCA  | GTGTTCGGAT  | TCTCATCGCC  | TCCAGTTTAC |
| 1851 | TAGTAATAAA  | GCAGCAGATG  | AAGGCGGGGG  | CCTGTATTGT  | GGTGACGATG |
| 1901 | TCAGCGTAA   | GAACCTTGACA | GGGAAAAACAC | TATTTCAGAA  | GAATGACATG |
| 1951 | GAGAAACATG  | GAGGTTGGCT  | CTCTCTCGCC  | TCAGGAAAAAT | CTCTGACTAT |
| 2001 | GACATCGTTA  | GAGAGCTTCT  | GCTTAAATGC  | AAATACAGCA  | AAGGAAACAG |
| 2051 | GAGGCGGGTG  | GAATGTCCCT  | GAATAATATTG | TACTCACACTT | CACCTATACT |
| 2101 | CCACCTTCAA  | ATGAACCTCG  | GCCTGTGACG  | CAGCCCGTGT  | ATGCGAGAGC |
| 2151 | CTCTTGTACT  | GGAAATACAG  | CCACAAAAAG  | TGGTGGGGGG  | ATTTTACAGC |
| 2201 | AAAAATGCGG  | CTCTTCAAA   | TTAATCTTCTG | TAACTTTTGA  | TCAAATATCC |
| 2251 | CTCTTCAGAA  | ATGGTGGTGC  | CTTACTTTACC | CAAAAAGGCT  | CAGATAAATC |
| 2301 | GGACGTGTCT  | TTGCACCTATA | TTACAATATGT | CAATATCACC  | AKCATATACG |
| 2351 | CTACAGGAA   | TGGTGGGGGG  | ATTGCTGGGG  | GAAGAAGACA  | TTTCGATCGC |
| 2401 | ATTGATTAAT  | TTACAGTCCA  | AAGCAACCAA  | GCAAAAGAA   | GTGCTGGGGT |
| 2451 | TTATCTTTGAA | GATGCCCTCA  | TCTTGGAAAA  | GGTATTATCA  | GGTCTGTCT  |
| 2501 | CACAAATATC  | AGCTACAGAA  | AGTGTGGGG   | GTATCTACGC  | TAAAGGATAT |
| 2551 | CACATACAA   | CTCTACTCGG  | AAGCTTCAGA  | ATTACCGATA  | ATPAACTGGA |
| 2601 | AACCTAGCTT  | ACTATACGCA  | CTAATTTATA  | TGGTGGGGGG  | ATTCTATCCA |
| 2651 | GTGGAGCTGT  | CACGCTAACC  | AAATATATCTG | GAACCTTTGG  | CAATACAGGA |
| 2701 | AACCTCTGTA  | TCAATACAGC  | GACATCCGAG  | GATGCAGATA  | TACAGGTTGG |
| 2751 | GGGCAATTAT  | GCAACCAAGT  | CTCTCTCAAT  | AAATCAATGT  | AATACACCCA |
| 2801 | TTCTATTTAG  | CAACAACTCT  | GCCTGCACTA  | AAAAAACATC  | AACAAACAA  |
| 2851 | CAAAATTCCTG | GTGGGGCTAT  | CTTCTCGCTT  | GCAGTAACTA  | TCGAGAAATA |
| 2901 | CTCTCAGCCC  | ATTATTTTCT  | TAAATATATC  | GCACAAAGTCG | GAAGCAACTA |
| 2951 | CAGCAGCAAC  | TGCAGGAAAT  | AAAGATATGCT | GTGGAGGAGC  | CATTGCAGCT |
| 3001 | AACCTCTGTA  | CTTTAACAAA  | TAAACCTGAA  | ATPAACTTTA  | GAAGAAATTA |
| 3051 | TGCAGAAAT   | GGAGGAGCGA  | TTGGCTGTAT  | TGAICTTACT  | AATGGCTCAC |
| 3101 | CTCCCCGTA   | AGTCTCTATT  | GCAGACACAG  | GTCTGTCTCT  | TTTTCAAGAC |
| 3151 | AACCTCTGCT  | TAAATCGCGG  | AGGGCGTATC  | TATGGAGAGA  | CTATCGATAT |
| 3201 | CTCCAGGACA  | GGTGCAGATT  | TCATCGGTAA  | CTCTTCAAAA  | CATGATGGAA |
| 3251 | GTGCAATTGT  | CTGTTCACAA  | GCCCTACTC   | TGGCGCCAAA  | CTCCCACTT  |
| 3301 | ATCTTTGAAA  | ACAAATAGGT  | TACGGARACC  | ACAGCCACTA  | CAAAAGCTTC |
| 3351 | CATAAATAAT  | TTAGGAGCTG  | CAATTTATGG  | AAATATATGAG | ACTAGTGACG |
| 3401 | TCACATATCT  | TTTATCAGCT  | GAGAAATGGA  | GTATTTTCTT  | TAAAAACAAT |
| 3451 | CTAATGACAG  | CAACAAACAA  | ATACTGTGAG  | ATTGCTGGAA  | ACGTAAATTT |
| 3501 | TACAGCAATA  | GAAGCTTCA   | CAGGGAAGAG  | TATATCTTTT  | TATGATGCGA |
| 3551 | TTAAAGCTTT  | CACCAAGAAA  | ACAAATGCTC  | AAGAGCTTAA  | ATTAAATGAA |
| 3601 | AAAGCGACAA  | GTACAGGAAC  | GATTTCTATT  | TCTGGGGAAC  | TTACAGGAAA |
| 3651 | TAAATCTTAT  | ATTTCACAGA  | AAGTCACTTT  | CGCACATGGG  | AATCTCAITC |
| 3701 | TAGGTAATAA  | TGCAGAACTT  | AGCGTAGTTT  | CCTTTCAACC  | ATCTCCAGGC |
| 3751 | ACCACATATCA | CTATGGGCC   | AGGATCGGTT  | CTTTCAACCC  | ATAGCAAGA  |
| 3801 | AGCAGGAGGA  | ATCGCTATAA  | ACAAATGCTAT | CATTGATTTT  | AGTGAATTCG |
| 3851 | TTCTACTATA  | AGATAATGCA  | ACAGTAGCTC  | CACCCACTCT  | TAAATAGTA  |
| 3901 | TCGAGAACTA  | ATGCAGATAG  | TAAAGATAAG  | ATTGATATTA  | CAGGAACTGT |
| 3951 | GACTCTTCTA  | GATCCTAATG  | GCACCTTATA  | TCAAAATCTC  | TATCTTGGTG |
| 4001 | AAGACCGCGA  | TATCACTCTT  | TTCAATATAG  | CAAAATCTGC  | AAGTGGGGCA |
| 4051 | GTTACAGCCA  | CGAATGTCA   | CCTTCAAGGG  | AATTTAGGAG  | CTAAAAAAGG |
| 4101 | ATATTTAGGA  | ACCTGGAATTT | TGGATCCAAA  | TCTCTCGGGT  | TCAAAAATTT |
| 4151 | TTCTAATAAT  | GACCTTTTAC  | AAATACCTGC  | GCTGGCCCTA  | CATCCCTAGA |
| 4201 | GACAACTACT  | TCTACATCAA  | CTCTATTGTT  | GGAGCAACAA  | ACTCTTTAGT |
| 4251 | GACTGTGAAA  | CAAGGGATCT  | TAGGGGAACAT | GTTTGAACAT  | GCAAGGTTTG |
| 4301 | AAGATCCTGC  | TTTCAACAA   | TTCTGGGCTT  | CGGCTATAGG  | ATCTTTCCCT |
| 4351 | AGGAAAGAG   | TATCTCGAAA  | TTCTGACTCA  | TTCACTATAC  | ATGGCAGAG  |
| 4401 | CTATACCGCT  | GCTGTGGATG  | CCAAACCTCG  | CCAGAAATTT  | ATTTTAGGAG |
| 4451 | CTGCTTTCAG  | TCAGGTTTCT  | GGTCAAGCGC  | AGTCTGAATT  | TCACCTTGAC |
| 4501 | AACATATAGC  | ATAAAGGCTC  | AGGTCACTCT  | ACACAGCAT   | CTCTTTATGC |
| 4551 | TGGCAATATC  | TTCTATTTTC  | CTGCGATACG  | GTCTCGGGCT  | ATCTGATTC  |
| 4601 | AAGGTGTGGC  | GACCTATGGT  | TATATGCAAC  | ATGACACCA   | AACCTACTAT |
| 4651 | CTCTTATATG  | AAGAAAAAAA  | TATGGCAAA   | TGGGATAGCA  | TTGCTTGGTT |
| 4701 | ATTTGATCTG  | GGTTTCAAGT  | TGGATCTTAA  | AGAACTTCAA  | CCTCACTCTA |
| 4751 | CAGCAAGGCT  | TACCTTCTAT  | ACAGAGAGCTG | AGTATACAG   | AATTCGCCAG |
| 4801 | GAGAAATTTCA | CAGAGCTAGA  | CTATGATCTC  | AGATCTTTCT  | CTGCATGCTC |
| 4851 | TTATGCGAAAC | TTAGCAATTC  | CTACTGGATT  | CTCTGTAGAC  | GGAGCATTAG |
| 4901 | CTTGGCTGGA  | GATTAATCTA  | TATAATTAAG  | TATCAGCTGC  | GTACCTCCCT |
| 4951 | GTGATTTCTCA | GGAAATATCT  | AAAAGCGACC  | TATGAATTTT  | TCTCTACAAA |
| 5001 | AGAAAAGGGC  | CAGCTAGTCA  | ACGTTCTCCC  | TACAGAAAC   | CGAGCTCGTG |
| 5051 | CAGAGGTGAG  | CTCTCAAAAT  | TATCTTGGAA  | GTATCTGGAC  | ACTCTACGGC |
| 5101 | ACGTATATCTA | TTGATGCTTC  | AATGAATACT  | TTAGTGCAAA  | TGGCCAGCGG |
| 5151 | AGGGATCCGG  | TTTGTATTCT  | AG          |             |            |

The PSORT algorithm predicts an outer membrane location (0.926).

The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 60A) or his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used in Western blot (Figure 60B) and FACS (Figure 60C) analyses.

- 5 The cp6830 protein was also identified in the 2D-PAGE experiment (Cpn0540) and showed good cross-reactivity with human sera, including sera from patients with pneumonia.

These experiments show that cp6830 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 61

- 10 The following *C.pneumoniae* protein (PID 4376854) was expressed <SEQ ID 121; cp6854>:

```

1  MSIAIARBOY AAILDMHPKP SIAMPSSEQA RTSWEKRAQH PVLVRLLEII
51  WGVVKKLLGL IFFIPLGLFW VLQKICQNF ILLGAGGWIFR PICRDSNLLR
101 QAYAAARLFA SFQDHVSVSR RVCLQYDEVF IDGLELRLPN AKPDRMMLIS
151 NGNSDCLBYR TVLQGEKDWI FRIAEESQSN ILIFNYPGVM KSQGNITRNN
201 VVKSQYQAVR YLRDEPAGPQ ARQIVAYGYS LGASVQABAL SKELADGSDS
251 VRFVVKDRG ARSTGAIVAKQ FIGSLGVMLA NLTHWNINSE KRSKDLHCPE
301 LPITYGKDSQG NLIGDGLFKK ETCFAAPFLD PKNLEECESG KIPVAQTGRLR
351 HDHILSDSVI KEVAGHIQRH PDN*
```

The cp6854 nucleotide sequence <SEQ ID 122> is:

```

20  1  ATGTCAATAG CTATTGCCAG GGAACAATAC GCAGCTATAT TGGATATGCA
51  TCCTAARACCT TCGATCGCCA TGTTTTCTTC GGAGCAGGGC AGAATCTTCT
101 GGGAGAAACG ACAGGCTCAT COTTAACCTTT ATCGTCTTCT TCGATATGCA
151 TGGGTTGTTG TGAAATTTCCT TCTCGGCTTA ATCTTCTTTA TTCCCTGGGG
201 TCTTTCTCGG GTCCCTTCAGA AGATATGTCA GAATTTTTAT CTCTCTGGTG
25  251 CAGGAGGGTG GATTTTTAGA CCCATATGCA GGGACTCTRA TTTATTTGCGA
301 CAAGCTTACG CCGCGCTCTT TTACTCCGCT TCATTTCCAAG ATCATGTCTC
351 CTCTGTGCGA AGGGTTTGCT TACAGTATGA CGAGGTCTTT ATTGACGGAT
401 TGGAGTTACG TCTTCCCAAT GCTAAGCCAG ATCGATGGAT GTTAATCTCC
451 AATGGAAACT CCGATTGCTT AGAGTATAGG ACAGTGTCTG AAGGGGAAAA
30  501 GGACTGGATA TTCCGTAATG CTGAAGAGTC TCAATGCCAAC ATTTAATCT
551 TCAATTACCC AGGAGTCATG AAGAGCCAAG GGAATATAAC AAGAAACAAT
601 GTAGTCAAAT CTATATCAAGC ATCGGTACGC TATCTTAGAG ATGAACCCGC
651 AGGACCTCAG GCGGCTCAAA TCGTTGCTTA TGGCTATTCT TTAGGAGCTA
701 GTGTCTCAGC CGAAGCATTA AGTAAAGAGA TCGCAGACGG AAGTATAGC
35  751 GTCCCTTGTT TTGTCGTATA AGATCGAGGA GCTCGCTCTA CAGGAGCCGT
801 TGCTAAACAG TTTATTGGAA GTCTAGGAGT TTGGCTGGCG AATCTTACCC
851 ATTTGGAATAT TAATTTTGAA AAGAGAAGCA AGGACTTGCA TTGCCAGAGA
901 CTCTTTATTT ATGGCAGAGG ATCCCAAGGT AATCTTATCG GGGATGGATT
951 GTTCAAAAAA GAGACCTGCT TCGCAGCACC ATTTTATGAT CCTAAAAAAT
40  1001 TGGAAAGAGT TTCAGGGAAG AAAATCCCTG TAGCTCAGAC CGGTCTAAGA
1051 CACGATCATAT TCTTTTCCGA TGATGTGATT AAGAAGTTG CAGGTCTATAT
1101 TCAAAGACAT TTCTGTAATT A
```

The PSORT algorithm predicts an inner membrane location (0.461).

The protein was expressed in *E.coli* and purified as a GST-fusion product, as shown in Figure 61A.

- 45 The recombinant protein was used to immunise mice, whose sera were used in Western blot (Figure 61B) and FACS (Figure 61C) analyses. A his-tagged protein was also expressed.

These experiments show that cp6854 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

## Example 62

The following *C.pneumoniae* protein (PID 4377101) was expressed <SEQ ID 123; cp7101>:

```

1  MYSCYSKGIS HNYLHPMSR LDIFVFDSL I ANQDONLLEE IPCSEDTVLF
51 KAYRTTALQS PLAANKNLNIA RKVANYILAD NGRIDTVKLK EAIHHLSQCT
101 YPLGPHRRNE AQDRBHLKLM LKALKENPKL KESIKTLFVP SYSTIQNLIR
151 LHTALNPOQT LSTIHVRQAA LTALEPTVLRQ DVGSCFATAP AILHQEYVPE
201 RFLKDLNDLI SSGKLSRIVN QREIAVPINL SGCIGELFKP LRILDLYPD
251 LVKLSSSPGL KKAFAANLI ETLGDSQAQI QQLLSHQVLM QKLQNVHBTL
301 TANDIIKSTL LHYVQLQEST VRAIPFKEGL FSKQVAFST QHPRELBIQ
351 RVYHYLVHAYE EAKSAFIHDT QNPLLKAWAY TLATLADASQ PTISNHIRLA
401 LGWKSIEDPHS LVSLVTHFVR REVENIRILV QQCQBTYHBA RSQLEYIEGR
451 MRNPLNNQDS QILTMHMRF RQELNKALEY WDSAQREKAKK FLHLPEFLSS
501 FYTKQIPLYP RSSYDAFIQE FAHLYANAPA GFRILPTHGR THPNWSPRIY
551 SINEFIRFLS EFFTSTESLE LGHVAVINLE KETSRLVHNI TAMLHTDVFQ
601 EALLTRILEA YQLFVPPSIL NHLDQLSQT P WVYVSGGTVD TLLLDYFESS
651 EPLTLTEKHP ENPHELAAPY ADALKDLPTG IKSYLEEGSH SLLSSSPTHV
701 FSIAGSPLF REAWNDNWSY YTWLRDVVVK QHQDPLQDTI LPQLSIIAFI
751 ENFCNKYALQ HVVVDPHDFC SDHSLTLPEL YDKGSRFLSS LPTKDKTVAL
801 IYIRRLLYLM VREVPPYVSEQ QLEPELVNVS SYLGISSRIT YEKFRSLIEE
851 TIPKMTLLSS ADLRHIYKGL LMQSQKIYVT EEDTYLRIT AMRHNLAIP
901 APLLFADSNW PSIIYPGPILN PGTEIDLAK FNYAGLGQGP LDNIQELFAT
951 SRPWLYANP IDYGMPPPPG YRSRLPKEFF *

```

The cp7101 nucleotide sequence <SEQ ID 124> is:

```

1  ATGTATTTCGT GTTACAGCAA AGGAATATCC CATAACTATC TTCTACATCC
51 TATGTCACGTT TTGGATATTT TGTGTTTCGA TTCTCTGATC GCAACACGAG
101 ATCAAAATCT CTCTTGAGAA ATTTTCTGTT CTGAAGACAC AGTTTATTAT
151 AAAGCCTACC GTACTACGGC TCTCAATACC CCTCTAGCTC CTAAGAACCT
201 AAATATCGCC CGTAAAGTCG CAATTTATAT CTTAGCTGAC AATGGGGA
251 TCGATACAGT AAAGCTTGTC GAAGCCATTC ACCATCTCTC ACAATGTACC
301 TATCCTTTAG GCCTCATCGC CCATATGAA GCTCAAGATC GTGAACACCT
351 CCTTAAATAG CTAAGAAGTC TAAAGGAAAT TCCTAAATTA AAGAAAGCA
401 TCAAAATCTC TTCTTGCCCT TCATACTCTA CAATCCAAAA CCTAATTCGC
451 CATACACTAG CATTGAAATC ACAGACAATT CTCTCTACGA TTCAATGTGCG
501 TCAAGCAGCA CTCACAGCGC TCTTCACTTA CCTTCGGCAA GATGTAGGTT
551 CCTGTTTTCG TACGGCTCCT GCCATCTCTA TTCACAAAGA ATATCCAGAA
601 CGATTCCTTA AAGATCTCAA TGAATCTCAT AGCAGTGGCA AACTCTCTAG
651 AATCGTAAC CAAGGGGAAA TTGCGGTTC TTAATAACCT TCGGGATGCA
701 TTGGAGAGCT ATTCAAAGCT TTAAGGATTC TAGATCTTTA TCTGTATCCT
751 CTGGTTAAGC TCTCTCATC TCCAGACTC AAAAAAGCCT TTTCTGCTGC
801 CAATCTTATT GAATCTCTTG GGGATTCTGA AGCACAATTC CAACAGTTGC
851 TCTCGCATCA ATATTGTATG CAAAATCTAC AAAATGTCCA TGAGACCTTA
901 ACTGCTAACG ACATTATCAA ATCGACACTT CTGCACTACT ATCAGCTCCA
951 AGAAAGTACT GTACAGACTA TTTCTCTCA AGAAGGGTTG TTACGACAA
1001 AACAAAGTGC ATTTCTGACG CAACACCCCA GAGAGCTCTC AGAATAACAA
1051 CGGGTATACC ACTACTTACA TGCCATGAA GAAGCAAAAT CTGCTTTTAT
1101 CCATGACACT CAAAATTCCT TACTGAAAG CTGGGAGTAT ACTTTAGCGA
1151 CTCTTGCGGA TGCTAGCCAA CCTACCATCT CTGTATCTCT TAGTTACACA
1201 TTAGATGAGA AAGTAGAAGA CCTCACAAGT CTGTATCTCT TAGTTACACA
1251 CTTTGTGTGA GAGGAAGTAG AAAACATCG AATTTTAGTC CAACATATGT
1301 AACAGACCTA TCACGAAGCA CGCTCCCAAC TAGAATAATAT TGAAGGGCGG
1351 ATGCGCAACC CACTAAATTA TCAAGACAAG CAGATTTTGA CGATGGATCA
1401 CATCGCCTTC CGTCAAGAAC TCAATAAAGC TCTTTATGAG TGGGATATGT
1451 CTCGAAGAAA GGCAAGAGAA TTCTACACTT TCTCTGAATT CTCTATTCT
1501 TTCTATACAA AGCAAAATCT CTATACTTT TGTGTATCTT ACATGCTCTT
1551 CATTCAGAAA TTTGCTCATC TCTATGCTAA TGCTCCGGCT GGCTTCCTGA
1601 TTCTTTTTCAC GATGAGGAGC ACCCATCCGA ACACATGGTC CCCCATCTAT
1651 TCGATTAAAT AATTATATCG TTTTCTTTCT GAATCTTTCA CTTCCACAGA
1701 GTCAGAACTT CTGGGGGAAC ATGCGCTGAT CAATTTAGAG AAGAANAACAT
1751 CTCGGCTCGT CCACAACATC ACTGCCATGC TACACAGACA TGTTTCCCAA
1801 GAAGCTCTCC TTACAAGAAAT TTAGAAGGCC TATCAGCTTC CTGTGCTCTC
1851 TCCATCTTAA AACCACTTAG ATCAGCTGTC ACAAACTCCC TGGGTTTATG
1901 TTTCTGAGAG AACAGTGGAC ACTCTCTTTT TGGATTATTT TGAAGCTCCA
1951 GAACCTCTGA CACTTACAGA AAGCATCTCT GAAAATCTCT ATGAGCTTGC
2001 AGCTTCTTAC GCAGAGGCC TTAAGATCTT CCTCACAGA ATTAAAGATT

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|      |            |            |             |             |             |
|------|------------|------------|-------------|-------------|-------------|
| 2051 | ATCTAGAAGA | AGGATCCCAC | TCTCTACTTA  | GCTCATCACC  | CACCCACGTT  |
| 2101 | TTCCTATAAA | TCCGAGGATC | TCCCTTATTT  | CGGAGAGCTT  | GGGATATAGA  |
| 2151 | TGGGTACAGC | TATACCTGGC | TTCGTGATGT  | CTGGGTGAAA  | CAACACCAAG  |
| 2201 | ATTTCCTTCA | AGATACTATA | TTACCTCAGC  | TAACTATCTA  | TGCTTTTACA  |
| 2251 | GAGAAITTTT | GIACACAAAT | TGCTTTGCAA  | CATGTAGTTC  | ATGACTTTTCA |
| 2301 | TGATTTCTGC | TCCGACCACT | CCCTTGACTCT | TCCGAGAGCTC | TATGACAAAG  |
| 2351 | GATCGGTTT  | TCTAAGCTCC | TTATATGACA  | AGATTAAGAC  | CGTACCTCTT  |
| 2401 | ATCTATATAC | GCCTGCTCTC | CTACCTTATG  | GTCCGTAAAG  | TCCCTATATG  |
| 2451 | TTCTGAAKAA | CAGCTTCCAG | ATGCTTTAGA  | TAACTGCTCT  | TCATATCTCG  |
| 2501 | GGATTTACCT | TGCTATATAC | TATGAGAAAT  | TCCGCTCCCT  | GATAGAGGAA  |
| 2551 | ACCATCCCTA | AAATGACCTT | ACTCTCTTCA  | GCAGACCTGA  | GGCATATCTA  |
| 2601 | TAAAGGCTCG | CTCATGCAAA | GTTATCAAAA  | GAATCTACACC | GAAGAAGATA  |
| 2651 | CGTACCTCTG | CTTACCACTG | GCAATGAGGC  | ATCATATCTT  | TGCTATATCC  |
| 2701 | GCTCCTTTCG | TCTTTGACAG | CAGTAAGTGG  | CCCTCTATTT  | ATTTTGGATT  |
| 2751 | CATCCTAAAT | CCAGGAACCA | CAGAGATCGA  | TCTTTTGAAA  | TTTAACTATG  |
| 2801 | CAGGCTTGCA | AGGACAGCTC | CTTGACAAAT  | TCCAGAGAGCT | GTTCGCAACG  |
| 2851 | TCAAGACCTT | GGACCTCTTA | TGCAAACTCT  | ATAGATATAG  | GCATGCCACC  |
| 2901 | GCTTCCAGGC | TACCGCAGCC | GCCTCCCTAA  | AGAAATTTTC  | TAG         |

The PSORT algorithm predicts a cytoplasmic location (0.206).

- 20 The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 62A) or his-tagged product. The proteins were used to immunise mice, whose sera were used in Western blot (Figure 62B) and FACS (Figure 62C) analyses.

This protein also showed good cross-reactivity with human sera, including sera from patients with pneumonitis.

- 25 These experiments show that cp7101 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 63

The following *C.pneumoniae* protein (PID 4377107) was expressed <SEQ ID 125; cp7107>:

|    |     |            |             |            |            |             |
|----|-----|------------|-------------|------------|------------|-------------|
| 30 | 1   | MSIVRNSALP | LPCLSRSETP  | KKVRSHMKFM | KVLTPWLYRK | DLWVTAFLIT  |
|    | 51  | AIPGPSAHTL | VDIAGEPRHA  | AQATGVSGDG | KIVIGMKVPD | DPFAITVGFQ  |
|    | 101 | YIDHGLQPLE | AVRPQCSVPY  | NGITPDGTVI | VGINVAIGMG | SVAVKMVNGK  |
|    | 151 | VSELPMLPDT | LDSVASVSA   | DGRVIGGNRN | IMLGASVAVK | WBDVITQLP   |
|    | 201 | SLPDMNMACV | NGISSDGSII  | VPMVMVDSMR | NTAVQWIGDQ | LSVIGTLGGT  |
|    | 251 | TSVASALSTD | GTUVVGGSEN  | ADQSHTAYAY | KNGVMISDGT | LGGFVSLAHA  |
| 35 | 301 | VSSDGSVIVG | VTSNBSHRYH  | AFQYADQGMV | DLGT.GGPRS | YAQGVSGDGK  |
|    | 351 | VIVGRAQVPS | GDWIAFLCP   | QAPSPAPVHG | GSTVVTQNP  | RGMDVINATY  |
|    | 401 | SSLKNSQQQL | QRLLIQHSAK  | VESVSSGAPS | FTSVKGAISK | QSPAVQNDVQ  |
|    | 451 | KGTFPLYSRQ | VHGNVQNQQL  | LTGAFMDWKL | ASAPKCGFKV | ALHYGSDQL   |
|    | 501 | VKRAALPYTE | QGLGSSVLSG  | FGGQVQGRYD | FWLGGTIVLQ | PFMGILQVLL  |
| 40 | 551 | SREGYSEKNV | RFPPVSYDSVA | YSAATSPMGA | HVFASLSPKM | STAATLGVER  |
|    | 601 | DLNSHIDEFK | GSVSAAGNFV  | LENTSVSVLR | PFASLAMYVD | VRQQQLVTLTS |
|    | 651 | VVMKQQLTIG | TLSLVSQSSY  | NLSF*      |            |             |

The cp7107 nucleotide sequence <SEQ ID 126> is:

|    |     |             |            |             |            |             |
|----|-----|-------------|------------|-------------|------------|-------------|
| 45 | 1   | ATGAGTATAG  | TCAGAAATTC | TGCAITGCCA  | CTTCCGTTGT | TAAACAGATC  |
|    | 51  | CGAAACCTTT  | AAAAAAGTTA | GGTCGCATAT  | GAAATTTATG | AAAGTCCCTTA |
|    | 101 | CTCCATGGAT  | TTATCTGAAA | GATCTTTGGG  | TAAACAGCAT | CTTACTTGACA |
|    | 151 | GCAATCCGAT  | GATCTTTTTC | ACATACTCTT  | GTTGATATAG | CAGGAGAACC  |
|    | 201 | TCCGCAATGCT | GCTCAAGCAA | CAGGAGTTTC  | TGGAGATGTA | AAAAATGTTTA |
|    | 251 | TAGGAATGAA  | AGTTCCGAGT | GATCCTTTTG  | CTATACCTGT | AGGATTTTCAA |
| 50 | 301 | TATATTGATG  | GGCAATTGCA | ACCTCTTAGAG | GCAATGCTCT | CTCAATGCTCT |
|    | 351 | TGTATACCTC  | AAAGGTATAA | CCCCGGACGG  | AACGGTTTAT | GTGGGTACAA  |
|    | 401 | ACTATGCCAT  | CGGGAATGGT | AGTGTTCGCT  | TGAAATGGGT | AAATGGCAAG  |
|    | 451 | GTTCCTGAAC  | TTCCCAATCT | CCCTGACACC  | CTCGATTCTG | TAGCATCTGGC |
|    | 501 | AGTTTCTGCA  | GATGGAAGAG | TGATTGGAGG  | GAATAGAAT  | ATAAATCTTTG |
| 55 | 551 | GGCTTCTGTG  | TGCTGTGAAA | TGGAGGAGAC  | ACGTGATTC  | ACAATCTCT   |
|    | 601 | TCTCTTCTCT  | ATGCTATGAA | TGCTTGTGTT  | AACCGAATTT | CTTCAGATGG  |

|    |      |     |     |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|----|------|-----|-----|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5  | 651  | TTC | TAT | TAA | TAT | G | T | A | G | G | A | C | C | A | T | G | G | T | A | G | G | A | A | T | A | C | C | G | C | A | G |   |   |   |   |   |
|    | 701  | T   | A   | C   | A   | A | T | G | G | A | T | C | A | G | C | T | C | T | G | T | T | A | T | T | G | G | A | A | C | T | T | T | A | G |   |   |
|    | 751  | A   | C   | T   | T   | G | T | T | G | T | A | G | T | C | A | A | T | C | T | C | A | C | A | G | A | T | G | G | C | A | T | T | T | A | G |   |
|    | 801  | T   | T   | C   | T   | G | A | A | A | T | G | C | A | T | T | C | A | G | A | C | T | A | T | G | C | T | T | A | A | A | A | C | G | G | T |   |
|    | 851  | T   | A   | T   | G   | A | G | C | G | A | T | A | G | A | C | C | T | G | G | A | G | G | T | T | T | T | T | T | T | T | T | T | T | A | C |   |
| 10 | 901  | G   | A   | T   | C   | T | T | C | A | G | T | T | C | T | G | A | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | A |   |   |
|    | 951  | T   | A   | G   | A   | T | A | T | C | A | T | G | T | T | C | A | A | T | A | T | G | T | A | G | T | A | G | A | T | T | T | T | T | T | A |   |
|    | 1001 | C   | T   | T   | T   | A | G | G | A | G | C | T | T | G | A | T | C | T | A | T | G | T | C | A | A | G | T | T | T | T | T | T | T | A | G |   |
|    | 1051 | G   | T   | A   | T   | T | T | T | G | T | A | G | A | C | A | C | A | T | G | A | T | C | A | T | T | T | T | T | T | T | T | T | T | T | A |   |
|    | 1101 | A   | T   | G   | T   | C | T | T | T | C | A | A | G | C | T | C | C | G | A | T | T | T | T | T | T | T | T | T | T | T | T | T | T | A |   |   |
| 15 | 1151 | T   | C   | G   | T   | A | A | C | T | A | G | A | T | C | C | A | T | G | T | G | A | T | T | T | T | T | T | T | T | T | T | T | T | A |   |   |
|    | 1201 | T   | C   | C   | T   | T | T | G | A | A | A | T | A | G | C | A | C | A | A | C | A | C | A | T | T | T | T | T | T | T | T | T | T | A | G |   |
|    | 1251 | T   | A   | G   | T   | G | C | A | A | A | G | T | T | G | A | A | A | G | T | T | C | A | G | A | G | C | A | C | T | T | T | T | T | A | G |   |
|    | 1301 | T   | G   | A   | A   | A | G | G | T | G | C | A | T | C | A | A | A | C | A | G | A | G | C | C | T | G | C | A | A | A | T | G | A | T | A | G |
|    | 1351 | A   | A   | A   | G   | G | A | A | G | T | T | T | A | A | G | T | T | A | C | C | G | T | T | C | C | A | A | T | G | T | T | T | T | A | G |   |
| 20 | 1401 | T   | C   | A   | G   | A | A | T | T | G | C | T | C | A | G | G | A | G | C | T | T | T | A | T | T | G | A | T | T | T | T | T | T | A | G |   |
|    | 1451 | C   | T   | A   | A   | A | T | G | C | G | C | T | T | A | A | A | G | T | A | C | T | C | A | C | T | C | A | T | T | T | T | T | T | A | G |   |
|    | 1501 | G   | T   | A   | G   | A | A | C | T | G | C | A | G | C | T | T | T | C | T | T | T | T | A | C | A | G | A | A | T | T | T | T | T | A | G |   |
|    | 1551 | C   | T   | T   | C   | C | A | G | G | T | T | T | G | A | G | G | A | C | A | T | T | C | A | A | G | C | T | T | T | T | T | T | T | A | G |   |
|    | 1601 | G   | A   | G   | A   | A | C | T | G | T | T | C | T | C | A | A | C | C | C | T | T | T | A | T | T | G | C | A | T | T | T | T | T | T | A | G |
| 25 | 1651 | A   | G   | T   | A   | G | A | G | A | G | T | G | T | A | T | T | C | T | G | A | A | G | A | A | T | T | T | T | T | T | T | T | T | A | G |   |
|    | 1701 | T   | T   | C   | T   | G | T | A | G | C | C | T | A | C | A | C | A | G | C | A | T | T | T | T | T | T | T | T | T | T | T | T | T | A | G |   |
|    | 1751 | C   | T   | C   | C   | C | T | T | A | A | G | C | C | T | A | A | T | G | A | T | A | C | A | C | A | T | T | T | T | T | T | T | T | A | G |   |
|    | 1801 | G   | A   | T   | C   | T | G | A | A | T | T | C | A | C | A | T | A | T | A | G | A | T | T | T | T | T | T | T | T | T | T | T | T | T | A | G |
|    | 1851 | A   | A   | A   | C   | T | T | T | T | G | T | T | G | A | A | A | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | A | G |   |
|    | 1901 | C   | T   | C   | T   | T | G | C | T | A | T | G | T | A | T | A | G | C | A | A | C | A | A | T | T | T | T | T | T | T | T | T | T | A | G |   |
|    | 1951 | G   | T   | A   | T   | T | T | A | G | A | T | C | A | C | A | A | C | A | A | T | T | T | T | T | T | T | T | T | T | T | T | T | T | A | G |   |
|    | 2001 | A   | A   | G   | T   | A | G | C | A | T | A | A | T | C | T | T | A | G | C | T | T | A | A |   |   |   |   |   |   |   |   |   |   |   |   |   |

The PSORT algorithm predicts an inner membrane location (0.100).

- 30 The protein was expressed in *E. coli* and purified as a GST-fusion (Figure 63A) or his-tagged product. The proteins were used to immunise mice, whose sera were used in Western blot (Figure 63B) and FACS (Figure 63C) analyses.

These experiments show that cp7107 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### 35 Example 64

The following *C. pneumoniae* protein (PID 4376467) was expressed <SEQ ID 127; cp6467>:

|     |                   |                   |             |             |              |
|-----|-------------------|-------------------|-------------|-------------|--------------|
| 1   | <u>MLRFAVFTIS</u> | <u>TLNLTISQCS</u> | PSQSSKGLFV  | VNMKEMPRGL  | DPGKTRLIAD   |
| 51  | QTLRMLHYEG        | LVEEHSQNGE        | IKFALAESVT  | ISEDGTRYFT  | KIKNILWSNG   |
| 101 | DPLFAQDFVS        | SWKEILLKEDR       | SSVLYAFLP   | IKMARAISFD  | TESPENLQVR   |
| 151 | ALURRLLRIQ        | LETPCAHFLH        | PLLAIFFPV   | HEVLNANIS   | FEEMPTCCGA   |
| 201 | FRPVSLLEKQL       | RLHLSKNPMY        | INKSIVKLIHK | ILVQPIISNAN | TAAILLFFKHKK |
| 251 | LQWQSPFWGE        | PIPEEL SASL       | HQIDQLFSLF  | GASTTWLLEFN | IQRKPWNNAIK  |
| 301 | LRKALLALID        | XDMLATKVYVQ       | GLAEPTDHLF  | HERLYPTGVY  | BRKQNERILL   |
| 351 | BAQQLFBEAL        | DELQMTREDL        | EKEPTLTSTF  | SFSGRLQGM   | LRBQKKVLVK   |
| 401 | PTPIIVGQEF        | PTIQKNFLBG        | NYSLFVNQWT  | AAPIDPMSTYL | MIFANPGGIS   |
| 451 | PYHLQDSHFQ        | PLLILKIQEH        | KKHLRWQLII  | BALDYLDHCH  | ILBPLCHPNL   |
| 501 | RIALNKNIKN        | PNLFVRRITS        | FRFISGL*    |             |              |

A predicted signal peptide is highlighted.

The cp6467 nucleotide sequence <SEQ ID 128> is:

|    |     |            |             |            |            |            |
|----|-----|------------|-------------|------------|------------|------------|
| 50 | 1   | ATGCTCCGGT | TCTTCGCTGT  | ATTATATCA  | ACTCTTTGGC | TCAATACCTC |
|    | 51  | AGGATGTTC  | CCATCCCAAT  | CCCTCAAGG  | AATTTTGTGG | GTAATATGGA |
|    | 101 | AGGAATGCC  | AGCCTCCCTG  | GATCCTGGAA | AACTCTGCTC | CATTGCAGAC |
|    | 151 | CAAACTCTAA | TGCGCTCATCT | ATATGAAGGA | CTCGTCGAAG | AACATCCCCA |
|    | 201 | AAATGAGAG  | ATTAAACCAAG | CCCTTGACGA | AAGCTACACC | ATCTCCGAAG |
|    | 251 | ACGGGACTCG | GTACACATTT  | AAATCAAAA  | ACATCTCTTG | GAGTACGGGA |
|    | 301 | GACCCTCTGA | CAGTCAAGA   | CTTTGTCTCC | TCTTGGAGG  | AAATCTAA   |

351 GGAAGATGCG TCCTCCGTAT ATCTCTATGC GTTTTACCT ATCAAAAATG  
 401 CTCGGCGCAAT CTTTGATGAT ACTGAGTCTC CAGAAAATCT AGGAGTCCGA  
 451 GCTTTAGATA AGCGTCACTC CGAAATTCAG TTAGAAACTC CCTGCGCGCA  
 501 TTTCTTACAT TTCTTGACTC TTCTTATTTT TTCCCGTGT CATGAAATCT  
 551 TGCAGAACTA TAGCACTCTT TTGGAAGAGA TGCCCATATC CTGCGGTGCT  
 601 TTCCGCGCTG TGCTCTAGA AAAAGGCTTG AGCTCCATC TAGAGAAAAA  
 651 CCTATGTAC CATATAAAAG GCGGTGTGAA ACTACATAAA ATTATTGTAC  
 701 AGTTTATCTC AAACGCTAAC ACTGCAGCCA TTCTATTCAA ACATAGAAAA  
 751 TTAGATTGGC AAGGACTTCC TTGGGAGGAA CCTATCCCTC CAGAAATCTC  
 801 AGCTTCTCTA CATCAAGATG ACCAGCTCTT TTCTCTTCG GCGCTTTCGA  
 851 CTACATGGTT ACTCTTTAAT ATCAAAAAAA AACCTTGGAA CAACTGCTAAA  
 901 TTACGCAAGG CATTGAGCCT TGCAATAGAC AAAGATATGT TAACCAAAGT  
 951 GGTATACCAA GGTCTTGCGA AACCTACAGA TCATATCCCT CATCCAGAC  
 1001 TTTATCCAGG GACCTATCCC GAACGGAAGA GACAAAACGA AAGAATTTCT  
 1051 GAGGCTCAAC AACTCTTTGA AGAAGCTCTA GACGAACTTC AAATGACACG  
 1101 CGAAGATCTA GAAAAGGAAA CTTTGACTTT CTCAACCTTT TCTTTTCTTT  
 1151 ACGGAAGGAT TTGCCAAATG CTAAGAGAAC ARTGGAAGAA AGTCTTAAAA  
 1201 TTTACTATCC CTATAGTAGG CCAAGAGTTT TTCACAATC AAAAAAAGTT  
 1251 CCTAGAGGGG AACTATTCCT TAACCGTGAA CCAATGACG GCACGATTTA  
 1301 TTGATCCGAT GTCTTATCTC ATGATCTTTG CCAATCTCG AGGAATTTCC  
 1351 CCTATCACC TCCAGAGATC ACACITTTCAA ACTCTTCTCA TAAAGATCAC  
 1401 TCAAGAACAT AAAAAACAC TACGAAATCA GCATTATTAT GAAGCCCTTG  
 1451 ACTATTTAGA ACATGTCTAC ATTCTCGAAC CACTATGTCA TCCAAATCTT  
 1501 CGAATTGCTT TGAACAAAAA CATTAATAAC TTTAATCTTT TTGTTGACG  
 1551 AACTTCAGAC TTTCTGTTTA TAGAAAAACT ATAG

The PSORT algorithm predicts an outer membrane lipoprotein (0.790).

The protein was expressed in *E. coli* and purified as a his-tag product and a GST-fusion protein, as shown in Figure 64A. The recombinant his-tag protein was used to immunise mice, whose sera were used in a Western blot (Figure 64B). The recombinant GST-fusion protein was also used to immunise mice, whose sera were used in a Western blot (Figure 64C) and for FACS analysis (Figure 64D).

These experiments show that cp6467 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 65

The following *C. pneumoniae* protein (PID 4376679) was expressed <SEQ ID 129; cp6679>:

1 MRKNLVLLAS LQLLSPTLSS CTHLGSSGSY HPKLYTSGSK TKGVIAMLPV  
 51 FHRPGKSLSP LPWNLQKGFPT REISKRFPYAS BKVLFIKHNA SPQTVSQSVA  
 101 PIANRLPETI TBQFLPAEFI VATELLEQRKT GKAGVDSVPT ASVRVRVPDI  
 151 RHHKLALIVY EIIECSQPLT TLVNDYHRYG WNSKHFDSPT MGLMHSLRFR  
 201 EVVAVREGVY CANYS\*

A predicted signal peptide is highlighted.

The cp6679 nucleotide sequence <SEQ ID 130> is:

1 ATGCGAAAAA TGTGTGTATT ATTGGCATCT TTAGGACTTC TATCCCAAC  
 51 CCTATCCAGC TGCACTCACT TAGGCTCTTC AGGAAGTTAT CATCCTAAGC  
 101 TATACACTTC AGGGAGCAAA ACTAAGGTGT TGAATTGCGAT GCTTCCGTGA  
 151 TTTCTATCGCC CAGGAAAGAG TCTTTGAACCT TTACCTTGGG ACCTCCAAGG  
 201 AGAATTTACT GAAGAGATCA GCAAAAGGTT TTATCTCTGG GAAAGGCTCT  
 251 TCCTGATCAA GCACAATGCT TCACCTCAGA CAGTCTCTCA GTTCTATGCT  
 301 CCGATTGCGA ATGCTCTAACC CGAAACAATT ATTGAGCAAT TTCTTCTCTG  
 351 AGAATTCAAT GTTGCTACAG AACTGTTAGA ACAAGAGACA GGGAAAGAG  
 401 CAGGTGTCGA TTCTGTAAAC GCGTCTGTAC GTCTTCCGCT TTGTGATATC  
 451 CGTCATCATTA AAATGAGCTCT CATTTATCAA GAGATTATCG ARTGACGCCA  
 501 GCCTTTAACT ACCCTAGTCA ATGATTATCA TCGCTATGCG TGGAACTCAA  
 551 AACATTTTGA TTCAACGCCC ATGGGCTTAA TGCATACCGC TCTTTCTCGC

601 GAAGTTGTTG CCAGAGTTGA GGGCTATGTT TGTGCTAACT ACTCGTAG

The PSORT algorithm predicts an inner membrane location (0.149).

The protein was expressed in *E. coli* and purified as a his-tag product (Figure 65A) and as a GST-fusion product (Figure 65B). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 65C) and for FACS analysis.

These experiments show that cp6679 is a surface-exposed and immunoreaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 66

The following *C. pneumoniae* protein (PID 4376890) was expressed <SEQ ID 131; cp6890>:

```

10      1  MQQLFCVCV FAMSCSAYAS PRQDPSVMK ETRFNYYGII VSGQFVVKRG
      51  SDGTITKVLK NGATLHEVYS GLLHGEITL TFPHTTALDV VQIYDQGRIV
      101 SRKTFVFNGL PSQELFNED GTFVLTWRPD NNDSDIITKP YFIETTYQGH
      151 VIEGYSYTFN GKYSSSIHNG EGVRSVFSNN NILLSEETFN EGVVVKYTFP
      201 YFNRPESIT HYNGQPHGL RLTYLQGGIP NTIEWRYGF QDGTTIVFKN
      251 GCKTSEIAYV RGVKBSLELR YNQEIVABE VSWRNDPLHG ERKIYAGGIG
      301 KHEWYYRGRS VSKAFERLIN AAG*

```

A predicted signal peptide is highlighted.

The cp6890 nucleotide sequence <SEQ ID 132> is:

```

20      1  ATGAAACAAT TACTTTTCTG TGTTTGCGTA TTTGCTATGT CATGTCCTGC
      51  TTACGCATCC CCACGACGAC AAGATCCCTC TGTATATGAAG GAAACATTCCT
      101 GAAATAATTA TGGCATTAAT GTTCCCGTC AAGAATGGGT AAGCGGTGGT
      151 TCTGACGGCA CCATCACCAG AAGTACTCAA AATGGAGCTA CCCTGCATGA
      201 AGTTATATCT GGAGGCTCC TTCTATGGGA AATTACCTTA ACGTTECCCC
      251 ATACCAACAGC ATTGACGCTT GTTCAATAT ATGATCAAGG TAGACTCGTT
      301 TCTCGCAAAA CTTTITTTGT GAACGGTCTT CCATCTCAAG AAGAGCTGTT
      351 CAATGAAGAT GGCACGTTTG TCCTCACACG ATGGCCGGAC AACACGACA
      401 GTGATACCAT CACAAAGCCT TACTTCTATG AAACGACATA TCAAGGGCAT
      451 GTCATAGAAG GAGATTATAC TTCTTTTAAT GGGAAATACT CTTCATCCAT
      501 CCACAAATGGA GAGGGAGTTC GTTCTGTGTT CTCCTCCAAT AACATCCTTC
      551 TTTCTGAAGA GACCTTCAAT GAAGGTGTCA TGGTGAATA TACCACATTC
      601 TATCCGARTC GCGATCCCGA ATCGATTACT CATTATCAAA ATGGACAGCC
      651 TCACGGCTTA CCGCTAACAT ATCTACAAGG TGGCATCCCC AATACGATAG
      701 AGGAGTGGCG TTATGCTTTT CAAGACGGAA CGACCATCGT ATTTAAAAAT
      751 GGTGTGAAGA CATCTGAGAT CGCTTATGTT AAGGGAGTGA AAGAAGGTTT
      801 AGAACTGCGC TACAATGAAC AGGAAATTTG AGCTGAAGAA GTTTCCTTGGC
      851 GTAATGATTT TCTGATGGA GAACGTAAAG TCTATGCTGG AGGAATCCAA
      901 AAGCATGAAT GGTATTACCG CGGAGATCTG GTATCTAAAG CCAAAATTCGA
      951 GCGCTTAAAT GCTGCAGGAT AG

```

The PSORT algorithm predicts an outer membrane location (0.940).

The protein was expressed in *E. coli* and purified as a GST-fusion product, as shown in Figure 66A. The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 66B) and for FACS analysis. A his-tagged protein was also expressed.

These experiments show that cp6890 is a surface-exposed and immunoreaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 67

The following *C. pneumoniae* protein (PID 6172323) was expressed <SEQ ID 133; cp0018>:



```

1  MKTSVSMLLA  LLCSSASSIV  LHAATTPLPN  EDGFIGBNGT  NTFSPKSTTD
51  AAGTTYSLSFG  EVLYIDPKGK  GSITOTCFVE  TAGDLFFLGN  GNTLKFLSVD
101  AGANLAVAFV  QGSKNLSFDD  FLSLVITFES  KSAVTGKGS  LVSLGAVQLQ
151  DINTLVLFNS  ASVEQGVVTS  QNSCLTQCIK  NSAIPTQVTS  SKKGGAISTT
5    GGLTIRNWLQ  TLKFNENKAV  TSGGALDLGA  ASTFANHEL  IFSQNTSGN
251  AANGGAINCS  GDLTPTDNTS  LLLQENSTWQ  DGGALCSGTQ  ISITGSDGIN
301  VIGNTSQSGK  GATSAASLKI  LQQQGGALPS  NNVTWATPEL  GGAIFINPQG
351  SLQLPTQGGD  IVFEGNQVTT  AENATTKRN  VHLSESTAKN  TGLAASQGH
401  YVFPDPTFTN  DTGASDNLRI  NEVSAQKLKS  GSIVFSGERL  STAEATAENL
10   451  TSRINQFVTL  VEGSLVLKQG  VTLITQGFQ  EPESITLLDL  GTSI*

```

A predicted signal peptide is highlighted.

The cp0018 nucleotide sequence <SEQ ID 134> is:

```

1  ATGAAGACTT  CAGTTTCTAT  GTTGITGGCC  CTGCTTTGCT  CGGGGGCTAG
51  CTCATTGTGA  TCCATGCCG  CAACCACTCC  ACTAAATCCT  GAAGATGGGT
15   101  TTATTTGGGGA  GGGCAATACA  AATACTTTTT  CTCGAAATC  TACAACGGAT
151  GCTGCAGGAA  CTACCTACT  TCTCACAGGA  GAGGTTCTGT  ATATAGATCC
201  GGGGAAGAGT  GGTTCAAATA  CAGGAACCTG  CTTTGTAGAA  ACTGCTGGCG
251  ATCTTACATT  TTTAGGTAAT  GGAATACCC  TAAAGTTTCT  GTCCGTAGAT
301  GCAGGTCGTA  ATATCGCGGT  TGTCTCARGTA  CAAGGAAGTA  AGAATTAAAG
351  CTTCCACAGAT  TTCTTTCTC  TGGTGATCAC  AGAATCTCCA  AAATCCCGCT
40   401  TTACTACAGG  AAAAGGTAGC  CTAGTCAGTT  TAGGTACGCT  CCAACTGCAG
451  GATATAAACA  CTCTAGTTCT  TACAAGCAAT  GCCTCTGTCT  AAGATGTTGG
501  CGTGATTAAA  GGAACCTCCT  GCTTGATTCA  GGGAAATCAA  AATAGTGCAG
551  TTTTGGGACA  AAATACATCT  TCGAAAAGAG  GAGGGGCGAT  CTCCACGACT
601  CAAGGACTTA  CCATAGAGAA  TAACTTTAGG  ACGTTAAAGT  TCAATGAAAA
651  CAAAGCAGTG  ACCTCAGGAG  GCGCCTTAGA  TTTAGGAGCC  GCCTCTACAT
701  TCACTCGGAA  CCATGAGTTG  ATATTTTCAC  AAAATAAGAC  TCTCGGGAGT
751  GCTCGAAATG  CGGGAGCCAT  AAATTGCTCA  GGGGACCITA  CAITTTACTGA
801  TAACACTTCT  TTGTTACTTC  AAGAAAATAG  CACAAATGAG  GATGGTGGAG
30   851  CTTTGTGTAG  CACAGGAACC  ATAAGCATT  CCGGTAGTGA  TTCTATCAAT
901  GTGATAGGAA  ATACTTCAGG  ACAAAAAGGA  GGAGCGACTT  CTCGACCTTC
951  TCTCAAGATT  TTTGGAGGGC  AGGGAGGCCG  TCTCTTTTCT  AATAACGTAG
101  TGACTCATGC  CACCCTCTTA  GGAGGTGCCA  TTTTATCAAA  CACAGGAGGA
1051  TCCTTGCAGC  TCTTCACTCA  AGGAGGGGAT  ATCGTATTCG  AGGGGAATCA
1101  GGTCACTACA  ACAGCTCCAA  ATGCTACCC  TAAGAGAAAT  GTAATTCCAC
35   1151  TCGAGAGCAC  CGCGAAGTGG  ACGGGACTTG  CTGCAAGTCA  AGGTAAACGT
1201  ATCTATTTC  ATGATCCCAT  TACCACCAAC  GATACGGGAG  CAACGGATAA
1251  CTTACGTATC  AATGAGGTC  GTGCAAAAT  AAAGCTCTCG  GGATCTATAG
1301  TATTTTCTGG  AGAGAGATTG  TCGACAGCAG  AAGCTATAGC  TGAAAATCTP
40   1351  ACTTCGAGGA  TCAACACGCC  TGTCACTTTA  GTAGAGGGGA  GCTTAGTACT
1401  TAAACACGGG  GTGACCTTGA  TCACACAGG  ATTCCTCGAG  GAGCCAGAA
1451  CCACGCTTCT  TTTGGACTGT  GGGACCTCAT  TATAA

```

The PSORT algorithm predicts outer membrane (0.935).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 67A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 67B) and for FACS analysis.

These experiments show that cp0018 is a surface-exposed and immunaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 68

The following *C.pneumoniae* protein (PID 4376262) was expressed <SEQ ID 135; cp6262>:

```

1  MKRLRLIAIV  LIALSIILIA  GGVVLLTVAI  PGLSSVISPP  AGMGACALGC
51  VMLALGIDVL  LKKREVPILV  ASVTTTPTGT  SPRSGISISG  ADSTIRSLPT
101  YLLDEGHFQS  MKRLRLIAIV  LIVFSIILIA  SGVLLTVAI  PGLSSVISPP
151  AGMGACALGC  VMLALGIDVL  LKKREVPILV  ASVTTTPTGT  SPRSGISISG
55   201  ADSTIRSLPT  YPLDEGHFQS  MKRLRLIAIV  LIVFSIILIA  SGVLLTVAI
251  PGLSSVISPP  AEMGACALGC  VMLALGIDVL  LKKREVPILV  PAPTPREVII

```

301 DDIDHSSIRL QQEAKAALAR LPEEMSAFEG YIKVVESHLE NMKSLPYDGH  
 351 GLEBKIKHQI RVRSSSLKAM VPETLDIRI FEEEEFFPLS ARKRLIDLAT  
 401 TLVERKILTE QLERNNLRKA FSYLYQDSIF KKILDNFEL AKRFPILLSKS  
 451 ICRVLLIFEN HEGVAKSLK KLMVYLERV TYSLQKSVR DTGMSAKKK  
 501 ILHGHFFPSL EDNKITMKE HAEMLBSLS YKVTPLALGD HVRVDPSPDP  
 551 KKWDLGCTPC RDALSEISRD BQWQKKAHLK HCESLYPQAR DRLDTQSSKE  
 601 NKELEKAEQ EYISMERVK KFLSERVQER IFAIKGLYPN ILEREETVIG  
 651 QRTVTFPTVQC TWASDLTDI LGRIBVSRE DWNCESCVK VLRSHBVMS  
 701 WRVKQSYGPK KKKFQDMGS LERFFTEHIE ELVLQKDYI KHLSPFKVN  
 751 NKKHVQYAKF RLKVLSEDL E GLAQTESAE SLTQEBLPI LATRGALEKA  
 801 VPKGSLCAL ASKAKPYVEE DPFQSDSTQ LRLALRLQE AKASLEEBIK  
 851 RFNSLENDIA EERLLKESK QTFERAOLGV LREIAVESTY DLRSLNWTWE  
 901 GTPSEKUYVF SMVLYNYNEE KRRAKTRLVE MTQRYRDFRM ALEAMQFNEE  
 951 ALLQEELSIQ APSE\*

15 A predicted signal peptide is highlighted.

The cp6262 nucleotide sequence <SEQ ID 136> is:

1 ATGAGGAAAC TTCTGATTCT TCGGATCGTT CTCATGACTT TGAGCATTTAT  
 51 TTGATTGCA GGTGGTGTGG TATGTCTTAC TGTAGCGATC CCTGGATTAA  
 101 GTTCAGTCAT TCTCTCCCGC GCAGGGATGG GTCCCTGTGC TTTGGGATGT  
 151 GTGATGCTTG CTTTAGGGAT CGATGTCTTT CTGAAGAAAC GAGAAGTCCC  
 201 TATAGTCTCT GCATCTGTAA CTACGACACC AGGAAGCTGGC AGCCCTAGAA  
 251 GTGGTATTTC TATTTCAGGA GCTGATAGCA CCATACGTTCT TCTTCTACG  
 301 TATCTCTGGC AGCAGGAGCA TCCACAAATCC ATGAGGAAC TCTGTATTCT  
 351 TCGCATCGTT CTCATAGTIT TTAGCATTA TTTGATGCA AGTGGTGTGG  
 401 TATTGCTTAC TGTAGCGATC CCTGGATTAA GTCCAGTCTT TCTTCCCGC  
 451 GCAGGGATGG GTCCCTGTGC TTTGGGATGT GTGATGCTGT CTTTAGGGAT  
 501 CGATGTCTTT CTGAAGAAAC GAGAAGTCCC TATAGTCTCT GCATCTGTAA  
 551 CTACGACACC AGGAAGTGGC AGCCCTAGAA GTGGTATTTC TATTTCAGGA  
 601 GCTGATAGCA CCATACGTTCT TCTTCTACG TATCCCTTGG ACAGGGGACA  
 651 TCCACAAATCC ATGAGGAAC TCTGTATTCT TCGGATCGTT CTCATAGTTT  
 701 TTAGCATTAT TTTGATGCA AGTGGTGTGG TATTGCTTAC TGTAGCGATC  
 751 CCTGGATTAA GCTCGATCAT TCTCTCCCA CGGAGATGG GTGCTTGTGC  
 801 TTTGGGATGT GTGATGCTTG CTTTGGGAT CGAGTCTCTT CTGAAGAAAC  
 851 GAGAAGTCCC TATAGTAGTT CCGCACCATA TTCTGAAGA AGTCGTGATA  
 901 GATGATATAG ATGAAGAGAG TATACGCTG CAGCAGGAAG CTGAAGCCGC  
 951 TTAGCAAGA CTCTCTGAGG AGATGAGTGT ATTGGAAGT TACATAAAG  
 1001 TTGTCGAGAG TCATTGAGAG AACATGAAA GCCTGCCCTA TGTATGGTAT  
 1051 GGGCTAGAAG AGAAACAGAA ACATCAGATA AGAGTGCCTA GATCTTCTTT  
 1101 GAAGGCTATG GTTCCAGAA TTTTAGATAT CAGAAGAATT TTTGAAGAAG  
 1151 AAGAGTTCTT TTTCTCTCA GCTCGCAAC GACTTATAGA TTTAGTACT  
 1201 ACTTTAGTAG AGAGAAAAAT TTTAACAGAG CACTGTAGC GCAATANTTT  
 1251 AAGGAAGCGC TTTCCTTATT TATATCAGGA CTCATTTTT AAAAATAATTA  
 1301 TTGTAACCT CGAGAAGTTA GCATGGAAT TTAGTATTT GAGTAATCTA  
 1351 ATTTCTCGAT TTACAAATTA TTTTGAATAT CATGACAGTA GTGTAGCAAA  
 1401 GAGCGCTGTA CACAAGAAAT CAGTGTACT GGAGAGAGTA ACTTATAGGA  
 1451 GTTTCACAAA AAGCTATAGA GATATAGGCA TGTCACTCGC AAGATGAAA  
 1501 ATCTTGCACG CCAACCCCTT TTTCTCTTG GAGGATAATA AAAAGACGAT  
 1551 AATGAAGAA CACGACAGAG TGCTTGAAG TCTCAGTAGC TATAGGAAGG  
 1601 TATTTTTCAG TCTATCTGAT GAGAAGCTTG TAGATACACC TAGCATCCA  
 1651 AAGAATATGG ATTTGTGAGG AATCCCTCTT AGGACCGCTG TGCTTGAGAT  
 1701 TCTCTGTGAT GAACGATGGC AGAAGAAAGC ACATCTAAGG CATCAAGAT  
 1751 CCTCTATAC GCAAGCTAGG GATCGTTTAA CAGACACAGG CTCTAAAGAA  
 1801 AATCAGAAAG AGTTAGAGAA AGCTGAACAA GAGTACATAT CTTCTGGGAA  
 1851 ACGGGTTAAA AATTTTGAGA TTGAGAGAGT ACAGGAGAGG ATACGGGCAA  
 1901 TTCAAAGCTT TATCTCTAAT ATCTCTGAGA GAGAAGAAAG AACACAGGT  
 1951 CAGGAGACTG TGACTCCAAAC TGTCAAAGG ACAGCGCTCT CATCCGATT  
 2001 AACAGATATT TTAGGAAGAA TAGAGGCTCT CAGTAGGGAG GATTAACAGA  
 2051 ATCAAGAGTC TTGTGTAATA GTCTTAAGAA GTCATGAGGT AGAATGAGC  
 2101 TGGGAGTCA AACAGAGATA TGGCCCTAAG AAAAAGAGAT TCCAGATCA  
 2151 AATGGGTCTT TTAGAGAGGT TTTTTCAGAA GCATATTGAA GAGTTAGAGT  
 2201 TATTACAGAA GGACTACTCT AAACACTTGT CTTATTPTAA AAAAGTAAC  
 2251 AATAAGAAAG AGGTTCAATA TGCAGAGTTT AGGTTGAAGG TTTTAGAGTC  
 2301 AGATTTAGAA GGGATTCTAG CTCAGACTGA GAGTCTCGAG AGTCTGTATA  
 2351 CTCAGAGAGA ACTTCCGATP CTGCGAACTC GGGAGCGCTT AGAGAAAGCT  
 2401 GTTTTCAAGG GGAAGTCTATG TTGCGCGCTA CCAAGCAAGG CAAAGACCTTA

2451 TTTTGAAGAG GATCCAGAT TCCAAGATC TGATACGCAA TTGCGAGCTC  
 2501 TGACTCTAAG GTTACAGAGG GCTAAGCAA GCCTGGAGAA AGAGATAAAG  
 2551 AGATTTTCAA ATCTTAGAGAA CGATATTGCA GAGGAAGAC GCCTCTTCAA  
 2601 AGAGAGCAAG CAGACGTTCC AAGAGCAGG TTTAGGGOTT CTCCGAGAAA  
 2651 TTGCGAGTCA GTCTACTTAT GATTTCGCTT CCTTACACAA TACATGGGAA  
 2701 GGGACCCAGC AGAGTAGAGAA GGCTATTATT AGCATGTATC TTAATTATTAT  
 2751 CAACAGAGAG AAGCGTAGGG CTAAACAAG ATTGGTTGAA ATGACACAGA  
 2801 GGTATAGAGA TTTTAAATGT GCCTTGAAG CTATGCAAGT TAATGAAGAA  
 2851 GCCCTTTTGC AAGAGGAAC CTCTATTCAA GCTCCAGTG AATTA

10 The PSORT algorithm predicts inner membrane (0.660).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 68A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 68B) and for FACS analysis.

These experiments show that cp6262 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 69

The following *C. pneumoniae* protein (PID 4376269) was expressed <SEQ ID 137; cp6269>:

1 MYQENLRLLR RLLYNSVQKS YADRLFSYBK TRMVDTPILI PWSEDKEKCA  
 51 EAEKAFLEQQ KILLDYGKSI FWLNENDEIN LNDPWSWGLN TVRTKRVQFE  
 101 VDDSRWNHKL VLIQKLEDDY EKLLBESSKE STEANKLLLS DLVDRLEDAK  
 151 TKPFLLKQEE VETRVKDLRA RYGGTVDPKQ DTEAKKKVEL EASLETFLDS  
 201 IBSELVQCLE DQDIYWKQD VKDLARTQBL EBQDIEAKRE EABEDLRSLN  
 251 ERLKKSCTML DRAKWHIENA EDSITFWTISQ IEMKDMKARL KILKEDITSV  
 301 LPEIDEIETC LSELEPLILT TRELLTRSYL KFKICSEPTLL KMTSPFENNI  
 351 YVQIEYVQLQ NLGFKLQGIS QRFPGKQDDF ANLEBQVALQ KKRRLRELTON  
 401 FEIQSFNFMK BDFKAAAKDL YIRSTABQKM NFDVFCMELF REYHEEVNKP  
 451 LLELMYNCAD SYRDAKKKLK SLRLDEKELL QKEIKKKEEYF QKKQQRHADR  
 501 SRHTTYQKLR IAEELALELK KKI\*

The cp6269 nucleotide sequence <SEQ ID 138> is:

1 ATGTACCAGG AGAATCTAAG ATTGTGGAA AGGCTTCTTT ATAATAGTGT  
 51 TCAAAAGAGC TATGCGGATC GGCCTGTITTC CTATGAAAAG ACAAGATGG  
 101 TGCACGATAC TCCGCTGATT CCTTGGGJAG AGGATTAAGGA AAAATGTGCT  
 151 GAAGCTGAGA AAGCTTTCTT AGAGCAACAG AAGATTCTCC TAGATTATGG  
 201 AAATCTATAT TTTTGGCTGA ATGAGAACGA TGAGATCAAT TTAAACGATC  
 251 CTTGGAGTTG GGGTCTTAAT ACGGTGAGGA CTAGGAAAGT ATTCCAAGAG  
 301 GTTGACGACA GTGAACGTTG GAATCAATAAG GTACTCATTC AAAAAGCTCGA  
 351 GGACGATTAT GAGAAACCTC TAGAGGAAAG TTCAAAGAG TCTACTGAAG  
 401 CAATAAGGAA GCTTTTATCT GACTTAGTAG ATCGCTTGA AGATGCTAAG  
 451 ACAAAATTTT TCCTGAAGAA ACAGGAGGAG GTGAGGACTC CGGTAAAGGA  
 501 TCTTAGAGCT CGATTGAGG GCACAGTAGA TCCTAGACAG GATACGGAAG  
 551 CTAAGAAGAA AGTCGAATTG GAGGCTAGCT TAGAAGACTT TTTAGATTCC  
 601 ATCGAATCAG AGCTAGTACA GTGTTTAGAA GATCAGATTA TATATTGGAA  
 651 AGAACAGGAT GTCAAAGATC TAGCACGTAC GCAGAGGOTC GAGGAACAAG  
 701 ATATTGAAGC GAAGAGGGAA GAAGCTGCCG AGACCTTAAG AAGTCTTAAT  
 751 GAGCGTTTAA AGAAGTCAAA AACTATGTTA GATAGGACTA AATGGCATAT  
 801 TGAATAATGCT GAGGACAGTA TTACCTGGTG GACTAGTCAG ATAGAATGA  
 851 AGGATATGAA AGCAAGACTG AAGATCTTAA AAGAAGATAT AACCAATGTT  
 901 CTACCTGAAA TAGATGAGAT TGAACGCTGT TTAGAGCTAG AGGAGCTTCC  
 951 TTTGCTTACG ACACAGGAACT TCTTAACCTAA GTCTACCTA AAGTTTAAAG  
 1001 TTTGTTCCGA AACCATATTA AAAATGACTT CTGTGTTTGA GAACATATTC  
 1051 TATGTTTCAGG ATACACGAGGT TCAGCTGCAG AATCTTAGGT TTAAGTTTAA  
 1101 AGGTATATCT CAGAGATTTCG GAAAGAAACA AGACGATTTT CGCAATCTAG  
 1151 AGGAACAGGT TGCTTTGCAAA AAGAAACGAC TCAGAGAGCT CACTCAGAT  
 1201 TTTGAAATAC AAGGATTCAT TTTTCAATGAAA GAGATTTTAA AGGCACGCCG  
 1251 TAAAGATCTT TATATAAGAA GTACAGCTGA ACARAAGATG AACTTTGATG  
 1301 TGCCCTTGCA TGGAGCTCTC CTAGGTATC ATGAGGAGGT CAACAAGCCG  
 1351 CTTCTTGAGT TGATGTACAA TTGTGCAGAC AGTTATAGAG ATGCTAAGAA

```

1401 AAAGCTTTGC TCTCTACGTC TTGATGAAAA AGAGTTATTA CAAAAAGAAA
1451 TCAAGAAAGA GGAATTTTAT CAAAAGAAAC AACAAAGGCA TGCAGATAGA
1501 TCACGTCATA CTACGTATCA AAGCTACGA ATTGCTGAAG AGCTTGCTCT
1551 TGAGCTGAAG AAGAAATCT AA

```

5 The PSORT algorithm predicts cytoplasmic location (0.412).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 69A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 69B) and for FACS analysis.

10 These experiments show that cp6269 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 70

The following *C. pneumoniae* protein (P1D 4376270) was expressed <SEQ ID 139; cp6270>:

```

1 MKIPLRLLLI SLVPTLSMSN LLGAATTEEL SASNSFDGTT STTFSSSKTS
51 SATDGTNYVF KDSVVIENVP KTGETQSTSC FKNDAAAGDL NFLGGGFSFT
101 FSNIDMTAS GAAIGSEAM KTVTLQSGFA LSFLKSPAST VTNGLGAINV
151 KGNLSLLNDL KVLIQDNFST GDGGALNCAG SLKIANKKSL SFIONSSSTR
201 GGAHTKNTLT LSSGGETLFO GNTAPTAAKG GGAIAIADSG TLSISGDSGD
251 IIFEGNTIGA TGTVSHSAID LGTSAKITAL RAAQHTIYF YDPTIVGST
301 SVADALNINS PDTGDNKEYT GTIVFSGEKL TEABAKDBKN RTSKLLQNV
351 FKNGTVVLKG DVVLSSANGFS QDANSKLMD LGTSLVANTE SIELTNLEIN
401 IDSLSRNGKKI KLSAATAQKD IRIDRPVVLA ISDESFYQNG FLNEDHSYDG
451 ILELDAGKDI VISADSRSID AVQSPYGYQG KWTINWSTD KKAIVSWAKQ
501 SPNPATAEQEA PLVFNLLWGS FIDVRSFQNF IELGTGAPY EKRFVWAGIS
551 NVLHRSRGREN QRKFRHVSQG AVVGASTRMP GGDLSLIGFPA QLFARDKDYF
601 MNTNFAKTYA GSLRLQHDAS LYSVSVILLG EGGLREILLP VYSKTLPCSP
651 YGQLSYGHTD HRMKTESLPP PPPTLSIDHT SWGGYVWAGE LGTRVAVENT
701 SGRGFFQEXT PFVKVQAVYA RQDSFVELGA ISRDPSDSLH YNLAIPLGIK
751 LEKRFAEQYY HVVAMYSPIV CRSNPKCTTT LLNSQGSWKT KGSNLARQAG
801 IVQASGFRSL GAAAELEFNF GFWEGRSSRS YNVDAAGSKIK F*

```

30 A predicted signal peptide is highlighted.

The cp6270 nucleotide sequence <SEQ ID 140> is:

```

1 ATGAAGATTC CACTCGCGTT TTTATTGATA TCATTAGTAC CTACGCTTTC
51 TATGTCGAAT TATTAGGAG CTGCTACTAC CGAAGAGTTA TCGGCTAGCA
101 ATAGCTTCGA TGGAACTACA TCAACAACAA CGTTTTCTAG TAAAACATCA
151 TCGGCTACAG ATGGCACCAA TTATGTITTT AAAGATTCTG TAGTTATAGA
201 AAATGTACCC AAAACAGGGG AAACCTCAGT TACTAGTTGT TTTAAAAATG
251 ACGCTGCAGC TGAGAGATCTA AATTCTTTAG GAGGGGGATT TTCTTTCACA
301 TTTAGCAATA TCGATGCAC CACGGCTTCT GGAGCTGCTA TTGGAAGTGA
351 AGCAGCTAAT AAGACAGTCA CGTTATCAGG ATTTTCGGCA CTTTCTTTTC
401 TTTAAATCCC AGCAAGTACA GTGACTAATG GATTGGAGAG TATCAATGTT
451 AAAGGGAAAT TAAGCCTATT GGATAATGAT AAGGTATTGA TTCAGGACAA
501 TTTCTCAACA GGAGATGGCG GAGCAATTTA TTGTGCAGCG TCCTTGAAGA
551 TCGCAAAACA TAAGTCCCTT TCTTTTATTG GAAATAGTTT TTCAACACGT
601 GCGCGGAGCA TCTATACCAA AAACCTCACA CTATCTCTCT GTGGGGAAC
651 TCTATTTTCA GGAATACAGC CGCCTACGGC TGCCTGTAAG GAGAGTGCTA
701 TCGCGATTGC AGACTCTGGC ACCCTATCCA TTTCTGGAGA CAGTGGCGAC
751 ATTATCTTTG AAGGCAATAC GATAGGAGCT ACAGGAACCG TCTCTCATAG
801 TGCTATTGAT TTAGGAACTA CGCTGAAGT AACTCGGTTA CGTCTGCGC
851 AAGGACATAC GATATATCTT TATGATCOGA TTACTGTAAAC AGGATCGACA
901 TCTGTGTCTG ATGCTCTCAA TATTAAATAG CCTGATACTG GAGATACAAA
951 AGAGTATACG GGAACCATAG TCTTTTCTGG AGAGAAGCTC ACGGAGCCAG
1001 AAGCTAAAAG TGAGAAGAAG CGCAGTCTTA AATTACTTCA AAAATTTGCT
1051 TTTAAAAATG GAGCTGTAGT TTTAAAAAGT GATGTCTGTT TAAGTGGCAA
1101 CGGTTTCTCT CAGGATGCAA ACTCTAAGTT GATATAGAT TTAGGAGCST
1151 CTTGGTTTGC AARACAGAAA AGTATCGAGT TAACGAATTT GGAAATTAAT
1201 ATAGACTCTC TCAGGAACGG GAAAAAGATA AAACCTCAGT CAGGCCACAG

```

-111-

|      |             |            |             |             |             |
|------|-------------|------------|-------------|-------------|-------------|
| 1251 | TCGAAAGAT   | ATTCGIATAG | ATCGTCCTGT  | TGTACTGGCA  | ATTAGCGATG  |
| 1301 | AGAGTTTFTA  | TCAAAATGGC | TTTTTGAATG  | AGGACCATTC  | CTATGATGGG  |
| 1351 | ATTCTTGAGT  | TAGATGCTGG | GAAAGACATC  | GTGATTTCTG  | CAGATCTCTG  |
| 1401 | CAGTATAGAT  | GCTGTACAAT | CTCCGTATGG  | CTATCAGGGA  | AAATGAGCGA  |
| 1451 | TCAAATTGGTC | TACTGATGAT | AAGAAAGCTA  | CGGTTTCTTG  | GGCAAGCAG   |
| 1501 | AGTTTAAATC  | CCACTGCTGA | GCAGGAGGCT  | CCGTTAGTGT  | CTAATCTCTT  |
| 1551 | TTGGGGTTCCT | TTTATAGATG | TTGGTTCCTT  | ACAAGATCTT  | ATAGAGCTAG  |
| 1601 | TGACTGAAGG  | TGCTCCTTAC | GAAGAACAGAT | TTTGGGTTCC  | AGGCAFTTCC  |
| 1651 | AAATGTTTTCG | ATAGGAGCGG | CTCGAAGAT   | CAAGGAGAAAT | TCCTCTCATGT |
| 1701 | GAGTGGAGGT  | GCTGTAGTAG | GTGCTAGCAC  | GAGGATGCGG  | GCTGGTGTAT  |
| 1751 | CCTTGCTCTCT | GGGTTTTGCT | CAGCTCTTTG  | CGCGTACAC   | AGACTACITTT |
| 1801 | ATGAATACCA  | ATTTCGCAAA | GACCTACGCA  | GGATCTTTAC  | GTTTGCAGCA  |
| 1851 | CGATGCTTCC  | CTATCTCTCT | TGGTGAGTAT  | CTTTTAGGAG  | GAGGGAGGAC  |
| 1901 | TCGCGGAGAT  | CCGTGTGCTC | TATGTTTCCA  | AGAATCTGCC  | GTGCTCTTTC  |
| 1951 | TATCGGCGAGC | TTAGCTACGG | CGATACGGAT  | CATCGCATGA  | AGACCGAGTC  |
| 2001 | TCTACCCCCC  | CCCCCCCCGA | CGCTCTCGAC  | GGATCACTACT | TCTTGGGGAG  |
| 2051 | GATATGCTCTG | GGCTGGAGAG | CTGGGAACTC  | GAGTTGCTGT  | TGAAAAATACC |
| 2101 | AGCGGACAGAG | GATTTTTC   | AGAGTACACT  | CATTGTGTAA  | AAGTCCMAGC  |
| 2151 | TGTTTACGCT  | CGCCAGGATA | CGTTTGTAGA  | ACTAGGAGCT  | ATCAGTCTGT  |
| 2201 | ATTTTACGTA  | TGCGCATCTT | TATAACCTTG  | CGATTCCTCT  | TGGAATCAGT  |
| 2251 | TTAGAGAAAC  | GGTTTGCAGA | GCAATATTAT  | CATGTGTATG  | CGATGTATTC  |
| 2301 | TCCAGATGTT  | TGTCGTAGTA | ACCCCAAATG  | TACGACTACC  | CTACTTTCCA  |
| 2351 | ACCAAGGAGG  | TTGGAAGACC | AAAGGTTTGA  | ACTTAGCAAG  | ACAGGCTGGT  |
| 2401 | ATTGTTTCAG  | CCTCAGGTTT | TGCAATCTTG  | GGAGCTCGAG  | CAGAGCTTTT  |
| 2451 | CGGGAACTTT  | GGCTTTGAAT | GGCGGGGATC  | TTCTCTGTAG  | TATATGTAG   |
| 2501 | ATGCGGGTAG  | CAAAATCAAA | TTTTAG      |             |             |

The PSORT algorithm predicts outer membrane (0.92).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 70A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot and for FACS analysis (Figure 70B).

The cp6270 protein was also identified in the 2D-PAGE experiment (Cpn0013).

These experiments show that cp6270 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 71

The following *C.pneumoniae* protein (PID 4376402) was expressed <SEQ ID 141; cp6402>:

|     |             |            |            |            |             |
|-----|-------------|------------|------------|------------|-------------|
| 1   | MNVADLLSHL  | ETLLSSKIFQ | DYDGNPLQVG | DPQTPVKRIA | VAVTADLEFI  |
| 51  | QKQVAAEENV  | LIVHHGIFWK | GMYPYITGMI | HKRIQLLIEH | NIQLIAYLHP  |
| 101 | LDIAHP*PLGN | NRVADLLNWH | DLKPPGSSLP | YLVGQGSFSP | IDIDSPTDLL  |
| 151 | QVYVAPLKG   | SALGGPSKVS | SALLISGGAY | RELSSAATSB | VDCPIITGNFD |
| 201 | EFAMSTALEB  | NINFLAPGHT | ATEKVGPKSL | AHLKSEFPIT | STPTIDTANP  |
| 251 | F*          |            |            |            |             |

The cp6402 nucleotide sequence <SEQ ID 142> is:

|     |             |             |            |             |             |
|-----|-------------|-------------|------------|-------------|-------------|
| 1   | ATGAATGTTG  | CGGATCTCCT  | TTCTCATCTT | GAGACTCTTC  | TCTCATCAAA  |
| 51  | AATATTTCAG  | GATTATGGAC  | CCAACGGACT | TCAAGTTGGA  | GATCCCCAAA  |
| 101 | CTCGGATAAA  | GAAATATGCT  | GTTGCACTTA | CCGACAGATCT | AGAAACCAT   |
| 151 | AAACAAGCTG  | TTGCGGCGGA  | AGCAAAAGCT | CTCATTTGAC  | ACCAACGGAAT |
| 201 | TTTTTTGGAAA | GATGATGCCCT | ATCCTATTAC | CGGCAATGATC | CATTAAGCGCA |
| 251 | TCCAATTACT  | AATAGAACAC  | AATATCCATC | TCAATGCGTA  | CCACCTTCCT  |
| 301 | TGGGATGCTC  | ACCCTACCTT  | AGGAATAAAC | TGGAGAGTTG  | CCCTGGATCT  |
| 351 | AAATTTGGCAT | GACTTGAAGC  | CCTTTGGTGT | TTCCCTCCCT  | TATTTAGGAG  |
| 401 | TGCAAGGCTC  | TTTCTCTCCT  | ATCGATATAG | ATTCTTTTCA  | TGACCTGTTA  |
| 451 | TCTCAATATT  | ACCAAGCTCC  | CTTAAAGAGA | TCTGCTTTGG  | GGGGCCCCCT  |
| 501 | TAGAGTCTCC  | TCAGCAGCTC  | TGATCTCAGG | AGGAGCTTAT  | AGAGAACTCT  |
| 551 | CTTGGCGAGC  | CACGTCCCAA  | GTCGATTTGT | TCAATCAGAG  | AAATTTTGAT  |
| 601 | GAACTTGCAAT | GTCGACAGC   | TCTAGAAAGC | AAATCAACTT  | TCTAGCAATT  |
| 651 | TGGACATACA  | GCCACAGAAA  | AAGTAGGTCC | AAATCTCTTT  | CGAGAGCAAT  |

701 TAAAAAGCGA ATTTCTCTATT TCCACAACCT TTATAGATAC GGCCAACCCC  
751 TTCTAA

The PSORT algorithm predicts cytoplasmic (0.158).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 71A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 71B) and for FACS analysis.

These experiments show that cp6402 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 72

The following *C.pneumoniae* protein (PID 4376520) was expressed <SEQ ID 143; ep6520>:

1 MKHYLSFSPS ADFFSKQGA I ETQVLFGERV LVKGSTCYAY SGLFHNELLW  
51 KPYFGHSFRS TLVPCTPEFH IHFNVSUVSV DAFLEFWGIP LPFGTLLHVN  
101 SQNTVIFPKD ILNHMTIWS SGTPOCDPRH LRRLNYNFFA ELLIKDADLL  
151 LNFYVWGR SVHESLEKPG VDCSGFINIL YQAQYNVPR NAADQYADCH  
201 WISSFENLPS GGLIPLYFPE EKRIHVMLK QDSSTLTHAS GGKKEVEYFI  
251 LEQDGKFLDS TYLFFRNQR GRAFFGIPRK RKAFL\*

The ep6520 nucleotide sequence <SEQ ID 144> is:

1 ATGAACACT ACCTATCMTT TTCTCCTTCT GCTGATTTTT TCTCTAAACA  
51 GGGTGCTATT GAACTCAGG TCCTTTTGG AGAGCGCCTC TTAGTCAAGG  
101 GGAGCACTGT CTATGCATAT TCCCAATFAT TCCCAATGCA GCTGTATGG  
151 AAGCCCTATC CAGGTCATAG CTTCGCTTCT ACCCTAGTCC CCGTCACTCC  
201 TGAATTTTCT ATCCATCCAA ATGTTTCTGT GGTTCCTOTG GATGCATTTT  
251 TAGATCCTTG GGGATCCCT CTTCCTTTTG GAACTTTACT CCATGTGAAT  
301 TCTCAAATA CGTATTTTTC CCCTAAGGAT ATTCCTCAAT ATATGAACAC  
351 CATCTGGGGC TCCGGCACAC CTCATCGCA TCCTAGACAT CTAAGTCGTC  
401 TAAATATATA CTCTTTTGTCT GAACCTTTAA TAAAGAACGC AGACCTTTTA  
451 CTGAACCTTC CCGATGTATG GGGAGGACGG TCTGTACACG AAGTCTGGA  
501 AAAGCCGGGT GTTGATGTGT CGGATCTTAT CAATATCCTT TACCAGGCAC  
551 AGGGATACAA CGTCCCTAGA AACGCTGCAG ATCAATATGC GGATTTGTAT  
601 TGGATCTCTA GCTTTGAGAA CCTCTCTTCT GGTGGGTATA TATTTCTTTA  
651 CCCTAAAGAA GAAAGCGTA TTTCCTCATGT TATGTTGAAA CAGGATAGTT  
701 CCACCTCAT TCAATGCTTCT GGTGGAGGGA AAAAGTGGGA GTATTTTCAT  
751 TTAGAACAAG ATGGGAAGTT TTTAGATTTC ACTTATCTAT TTTTGTAGAA  
801 TAATCAGAGG GGACGGGCAT TTTTGGGAT CCCTAGAAAA AGAAAGCCT  
851 TCTGTAA

The PSORT algorithm predicts cytoplasmic (0.265).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 72A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 72B) and for FACS analysis.

These experiments show that cp6520 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 73

The following *C.pneumoniae* protein (PID 4376567) was expressed <SEQ ID 145; cp6567>:

1 MTSPIPFQSS GDASFLEAQP QQLPSTSESQ LVTQLLTMK HTQALSETVL  
51 QQQRDLPTA SIILQVGGAP TGGAGAPFQP GPADDIHHPI PPFVVPAQIE  
101 TEITTIIRSEL QLMRSTLQGS TKGARTGLV VTAIIMTISL LAIIIIILAV  
151 LGFTGVLPOV ALLMQGETNL IWMVSGSLX CFIALIGTLG LILTNKNTPL

201 PAS\*

The cp6567 nucleotide sequence &lt;SEQ ID 146&gt; is:

1 ATGACCTCAC CGATCCCCCT TCAGTCTAGT GCGGATGCCT CTTTCCTTGC  
 51 CGAGCAGCCA CAGCAACTCC CGTCTACTTC TGARTCTCGA AACGTAACCT  
 101 AATTCGTAAAC CATGATGAAG CATACTCAAG CATATCTCGA AACGGTTCTT  
 151 CAACAACAAC CGGATCGATT ACCAACCGCA TCTATTATCC TTCAAGTAGG  
 201 AGGAGCTCCT ACAGAGGAG CGGGTCGCCG TTTTCAACCA GGACCGGCAG  
 251 ATGATCATCA TCATCCCATC CCGCGCGCTG TTGTACCAGC TCAAAAGAA  
 301 ACAGAAATCA CCACTACAAG ATCCGAGTTA CAGCTCATGC GATCTACTCT  
 351 ACAACAAGGC ACAAAAGGAG CTCGTACAGG AGTCTAGTG GTTACTGCAA  
 401 TCTTAATGAC GATCTCCTTA TTGGCTATT TATCATATAT ACTAGCTGTG  
 451 CTTGGATTTA CGGGCGTCTT GCCTCAAGTA GCTTTATTGA TGCAGGTGA  
 501 AACAAATCTG ATTTCGGCTA TGGTGAGCGG TCTTATTATT TGCTTTATTG  
 551 CGCTAATTGG AACTCTAGGA TTAATTTTAA CAAATAAGAA CACGCCCTCA  
 601 CCGGCTTCTT AA

The PSORT algorithm predicts inner membrane (0.694).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 73A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 73B) and for FACS analysis.

20 These experiments show that cp6567 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 74

The following *C.pneumoniae* protein (PID 4376576) was expressed <SEQ ID 147; cp6576>:

1 MLINRNKVL QISTLALIQT PLTLESTKRV KRGHVVDGSI FLITEGENAS  
 251 NKHPLEPKLT RSGALFSQLD FDEDLRLAK EYDSVREKVE FSEKGNIAL  
 101 HLIAPKPIRN IHISGNQVVE RHKILKTLQI YRNDLPREK PLKGLDLRLT  
 151 YYLKRGFYAS SVDSLEBRQ EKGHIDVLIK INEFGCGKIK QLTFSGISRS  
 201 EKSDIQEFIQ TKQSHTTTSW PFGAGLXHPD IVEQDSLAI TLNHNHGYAD  
 251 AIVNSHYDLQ DKGNIILYMD IDRGSRVYTLG HVHITQGFVVL PKRLIRKQSQ  
 301 VGFNDLYCPD KINDGAHKIK QTYAKYGYIN TNVDVLPFIH ATRP IVDVTVY  
 351 EVSEGSYPYKV GLIKITGNTH TKSVDVJHRT SLFPGDTFMR LKLRDTEQRL  
 401 RNTGYQYQSVS VYTVRSQLPD MGNADQYRDI FVEVKEPTTG NLGLFI,GFSS  
 451 LDMLPGGIEL SRSNFDLFGA RNIFSEGFRC LRGGGEHLFL KANFGDKVTD  
 501 YTLKWKPKFH IINTPWLIGIE LDKSINRLS KDYAVQTVGG NVSTYIILNE  
 551 HLKYLGFYRG QQTSLHEKRK PLLGNIDSN KGFVSAAGVN LNYDSVDSPR  
 601 TPTTGIRGGV TFEVSGI,GGT VHYPTKLELNS SIYRKLTRKG ILKIKGEAQF  
 651 IKPYSNTTAE GVPVSEKFFL GGFVTVRGYK SPIIGPKYSA TEPQGLSLSL  
 701 LTSEEPYVPL TRQPNISAFV PLDSFVGLQ EYKISLKLRL SAGPGLRFD  
 751 VMNNVFMVLG FGWFRPTET LNGEKLDVQS RFFPALGGMF \*

40 A predicted signal peptide is highlighted.

The cp6576 nucleotide sequence &lt;SEQ ID 148&gt; is:

1 ATGCTCATCA TCGAATAAA AGTTATCTTG CAAATATCTA TTCTAGCGTT  
 51 AATCCAACCC CTTTAACTT TATTTTCTAC TGAAAAGGTT AAAGAAGGCC  
 101 ATGTGGTGGT AGACTCTATC ACAATCATAA CGGARGGAGA AATGCTTCA  
 151 AATAACAATC CTTTACCCTA ATTAAGACCC AGAAGTGGGG CTCTTTTTC  
 201 TCAATTAGAT TTTGATGAAG ACTTGAGAAT TCTAGCTAAA GAATACGACT  
 251 CTGTTGAGCC TAAAGTAGAA TTTCCTGAAG GGAAAACATA CATAGCCCTT  
 301 CACCTAATAG CTAACCCCTC AATTCGAAAT ATTCAATCTC CAGGAATCA  
 351 AGTCGTTCTT GAACATAAAA TTCTTAAAGG CCTACAATTT TACCGTAATG  
 401 ATCTCTTTGA ACGAGAAAAA TTCTTAAAGG GTCTGATGTA TCTAAGAAAGC  
 451 TATATCTTCA AGCGAGGATA TTTCGCAATC AGGTAGACTC ACAGTCTGGA  
 501 ACACAATCAA GAAAAGGTC ACATCGATGT TTTAATTTAA ATCAATGAAG  
 551 GTCTCTGCGG GAAATTTAAA CAGCTTACGT TCTCAGGAAT CTCTCGATCA  
 601 GAAAAATCAG ATATCCAAGA ATTTATTCRA ACCAGCAGC ACTCTACAAAC

|    |      |            |             |             |            |             |
|----|------|------------|-------------|-------------|------------|-------------|
|    | 651  | TACAAGTTGG | TTTACTGGAG  | CTGGAGCTCTA | TCACCCAGAT | ATTGTTGAAC  |
|    | 701  | AAGATAGCTT | GGCAATTACG  | AAATTACCTAC | ATAAFAACGG | GTACGCTGAT  |
|    | 751  | GCTATAGTCA | ACTCTCACTA  | TGACCTTGAC  | GACAAAGGGA | ATATTCTTCT  |
| 5  | 801  | TTACATGAGT | ATTGATCGAG  | GGTTCGGGATA | TACCTTAGGA | CACGTCCATA  |
|    | 851  | TCCAAAGGTT | TGAGGTTTTG  | CCAAAACGCC  | TTATATGAAA | GCAATCCCAA  |
|    | 901  | GTCCGGCCCC | ATGATCTTTA  | TTGCCCCGAT  | AAAATATGGG | ATGGGGGCTCA |
|    | 951  | TAAAGATCAA | CAAACTTATG  | CAAGATATGG  | CTACATCAAT | ACCAATGTAG  |
|    | 1001 | ACGTTCTCTT | CATCCCTCAC  | GCAACCCGCC  | CTATTATAGA | TGTAACCTAT  |
| 10 | 1051 | GAGGTAAAGT | AAGGGTCTCC  | TTATTAAGTT  | GGGTTAATTA | AAATTACTGG  |
|    | 1101 | GAATACCCAT | ACAAAATCTG  | ACGTTAATTT  | ACACGAAACC | AGTCTCTTCC  |
|    | 1151 | CAGGAGATAC | ATTCAAATCG  | TTAAAGCTAG  | AGAATACCTA | GCAACGTTTA  |
|    | 1201 | AGAAATACAG | GCTACTTCCA  | AAGCGTATGT  | GTCTATACAG | TTCGTTCTCA  |
|    | 1251 | ACTTGATCCT | ATGGGCAATG  | CGGATCAATA  | CCGAGATATT | TTTGTAGAAG  |
|    | 1301 | TCAAAAGAAC | AACAACAGGA  | AACCTAGGCT  | TATTCCTAGG | ATTTAGTTCT  |
| 15 | 1351 | CTTGACAATC | TTTTTGGAGG  | AACTGAACTA  | TCAGAAAGTA | ATTTTGATCT  |
|    | 1401 | ATTGAGAGCT | AGAAATATAT  | TTTCTAAGGG  | TTTTCTGTGT | CTAAGAGGCG  |
|    | 1451 | GTGGAGAAC  | TCTATTCTTA  | AAAGCCAACT  | TCGGGAGCAA | AGTCACAGAC  |
|    | 1501 | TATACTTTGA | AGTGGACCAA  | ACCTCATTTT  | CTAAACACTC | CTTGGATTTT  |
| 20 | 1551 | AGGAATTGAA | TTAGATAAAT  | CAATTACACG  | AGCATTTATC | AAAGATTATG  |
|    | 1601 | CTGTCCAAAC | CTATGGCGGG  | AACGTCAGCA  | CAACGTATAT | CTTGAAACGA  |
|    | 1651 | CACCTGAAAT | ACGGTCTATT  | TTATCGAGGA  | AGTCAAACGA | GTTTACATGA  |
|    | 1701 | AAAACGTAAG | TTCCTCTAGT  | GGCCAAATAT  | AGACAGCAAT | AAAGGATTGG  |
|    | 1751 | TCTCTGCTGC | AGGTGCTCAAC | TTGAATTACG  | ATCTGTGAGA | TATGCTCTAGA |
| 25 | 1801 | ACTCCAACCT | CAGGGATCTG  | CGGGGGGGTG  | ACTTTTGAGG | TTTCTGTTTT  |
|    | 1851 | GGGAGGAAGT | TATCATTTTA  | CAAAACTCTC  | TTTAAACAGC | TCTATCTATA  |
|    | 1901 | GAAACTTAC  | CGTAAAGGTT  | ATTTTGAAAA  | TCAAAGGGGA | AGCTCAATTT  |
|    | 1951 | ATTAAACCTT | ATAGCAATAC  | TACAGCTGAA  | GGAGTTCCTG | TCAGTAGAGC  |
|    | 2001 | CTTCTTCTTA | GGTGGAGAGA  | CTACAGTTTC  | GGGATATATA | TCCTTTATTA  |
| 30 | 2051 | TCGGTCCAAA | ATACTCTGCT  | ACAGAACCTC  | AGGAGGAGCT | CTCTTCGCTC  |
|    | 2101 | CTTATTTCAG | AAGAGTTTCA  | ATACCTCTCT  | ATCAGACAAC | CTAATATTAG  |
|    | 2151 | TGCTTTTGTA | TTCTTAGAGT  | CAGGTTTTGT  | CGGTTTACAA | GAGTATAAGA  |
|    | 2201 | TTTCGTATAA | AGATCTACGT  | AGTAGTGCTG  | GACTTGGTCT | CGGCTTCGAT  |
|    | 2251 | GTAATGAATA | ATGTTCTCTG  | TATGTATAGGA | TTTGGTTGCG | CCTTCCGTC   |
|    | 2301 | AACCGAGACT | TTGAATGGAG  | AAAAAATTGA  | TGTAATTCAG | CGATCTCTCT  |
| 35 | 2351 | TTCCTTAGG  | GGGCTATGTC  | TAA         |            |             |

The PSORT algorithm predicts outer membrane (0.7658).

The protein was expressed in *E. coli* and purified as GST-fusion (Figure 74A), his-tag and his-tag/GST-fusion products. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 74B) and for FACS analysis (Figure 74C).

40 The cp6576 protein was also identified in the 2D-PAGE experiment (Cpn0300).

These experiments show that cp6576 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 75

The following *C. pneumoniae* protein (PID 4375607) was expressed <SEQ ID 149; cp6607>:

|    |     |            |             |            |            |            |
|----|-----|------------|-------------|------------|------------|------------|
| 45 | 1   | MNKRQDKLK  | ICVLIISTLLI | VGIPARAPRG | DTFKTFLXSE | EALIIYNQCN |
|    | 51  | EDMRKELCDA | IEHADEEIPF  | RIYNLSBPFI | QQLSTRQAQA | KNKVITYYQK |
|    | 101 | FKIPQILKQA | SNVTLVEQPP  | AGRKLMDHQA | LSIDKDAWL  | GSANYTNLSL |
|    | 151 | RLDNNLILGM | HSSELCDLII  | TNTSGDPSIK | DTGKYPVLP  | QDRKIAIQAV |
|    | 201 | LEKIQTAQKT | IQVAMFALTH  | SEIIQALHQA | KQRGIHVDII | IDRSHSKLTF |
| 50 | 251 | KQLRQLNINK | DFVSNTPAC   | TLHHKFAVID | NKTLLAGSIN | WSKGRPSLND |
|    | 301 | ESLIIENLFT | KQONQKLEMI  | WKDLKKHSEH | PVTVDDEKRI | IEKSLPVEBQ |
|    | 351 | ENA*       |             |            |            |            |

A predicted signal peptide is highlighted.

The cp6607 nucleotide sequence <SEQ ID 150> is:



1 ATGAATFAAAA GACAAAAGGA TAAATFAAAA ATCTGTGTTA TTATTAGCAC  
 51 GTTGATTTTA GTAGGAATTT TTGCAAGAGC TCCTCGPGGT GACACTTTTA  
 101 AGGACATGTC GTAAATTTCT ATGCGATGCT ATAGAACACG CTGATGAAGA  
 151 GAGGACATGC GTAAATTTCT ATGCGATGCT ATAGAACACG CTGATGAAGA  
 201 GATCTTCGTA CGTATTATTA ACCTCTCAGA ACCCAAGATC CACCAAGGTT  
 251 TAATCTGACA AGCTCAAGCA AAAAACCAAG TTACGATCTA CTATCAAAAA  
 301 TTTTAAATTC CCCAAATCTT AANGCAGGCC AGCAATGTAA CTATTATGGA  
 351 GCACCTCCCA GCAGGGCGTA AACTGATGCA TCAAAAAGCT CTTTCCATAG  
 401 ATANGAAGA TGCTTSGGCTA GGAATCTGCA ACTACACCAA TCTTCTCTTA  
 451 CGTTTAGATA ATAACTTCAT TCTAGGAATG CATAGCTCGG AGCTCTGTGA  
 501 TCTCATTTAT ACAATATACCT CTGAGGACTT TTCTATAAAG GATCAACACG  
 551 GAAAGATTTT TGTCTTCCCT CAGATCGTA AAATGCAAT ACAAGCTGTA  
 601 CTCGAAAAAA TCCAGACAGC TCAGAAACCC ATCCAGTGTG CTATGTTTGC  
 651 TCTGACCCAC TCGAGATTA TTCAAGCCTT ACATCAAGCA AAACAACGAG  
 701 GAATCCATGT AGATATTATC ATGATAGAAA GTCATAGCAA ACTTACTTTT  
 751 AAGCAATTAC GACAATTAAA TATCAATAAA GACTTTGTTT CTATAAATAC  
 801 CGCACCCATG ACTCTTCACC ATAGATTTGC AGTTATAGAT AATAAACTC  
 851 TACTTGAGAG ATCTATAAAT TGGTCTAAG GAAGATTCTC CTAAATGAT  
 901 GAAAGCTTGA TCTACTTGA AAACCTGACC AAACAACAAA ATCAGAAACT  
 951 TCGAATGATT TGGAAAGATC TAGCTAAGCA TCCAGAACAT CCTACAGTAG  
 1001 ACAGTGAAGA AAAAGAAATT ATGAAAAAAA GTCTTCCAGT AGAAGACGAA  
 1051 GAAACAGCGT GA

The PSORT algorithm predicts periplasmic (0.934).

The protein was expressed in *E. coli* and purified as a his-tagged product (Figure 75A) and also as a  
 25 GST-fusion. The GST-fusion protein was used to immunise mice, whose sera were used in a Western  
 blot (Figure 75B) and for FACS analysis.

These experiments show that cp6607 is a surface-exposed and immunoaccessible protein, and that it  
 is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 76

30 The following *C. pneumoniae* protein (PID 4376624) was expressed <SEQ ID 151; cp6624>:

1 MDAKMYIFK VMRWIFCFVA CGITFGCTNS GFQNASRNC ILSMNRMIHD  
 51 CVERVVGNRL ATAVLIRGSL DPHAYEMVKG DKDKIAGSAV IPCNGLGLEH  
 101 TLSLRKHLEN NPNSVKLGER LIARGAFVPL EEDGICDPHI WMDLSIWKRA  
 151 VIEITVELIR KFPWNSAEFK ANSEELVCEM SILDSNAKQC LSTIPENRLY  
 201 LVSHGNAPSV PTRRYLATPE EVASGAMRSR CISPGLSPPE AQISVRDIMA  
 251 VVDYINEHDV SVVFPEDVLN QDALKKIVSS LKKSHLVRLA OKPLYSDNVD  
 301 DNYFSTFKHN VCLITEELGG VALEBQR\*

The cp6624 nucleotide sequence <SEQ ID 152> is:

1 ATGGATGCGA AATGGGATA TATATTAAAA GTGATGCGTT GGATTTTCTG  
 40 51 TTTCGTGGCA TGTGGTATTA CTTTTGGATG TACCATTCTT GGGTTTCAGA  
 101 ATGCAAAATC AGCTCTCTGT ATACTATCCA TGAATCGCAT GATTCATGAT  
 151 TGTGTTGAAA GAGTCGTGGG GAATAGGCTT GCTACCCGTG TTTTGTACAA  
 201 AGGATCCTTA GACCTCTATG CGTATGAGAT GGTATAAGGG GATAAGGACA  
 251 AGATTGTCTG AAGTGGCCGT ATTTTGTGTA AGCGCCGTGG TCTTGAGCAT  
 301 ACATTAAAGT TCGGGAAGCA TTTAGAAAAT AATCCCAATA GTGCAAGGTT  
 351 AGGGGAGCGG TGTATAGCGC GTGGGCCCTT TGTTCCTCTA GAAGAAGACG  
 40 401 GTATTTCGCA TCTCATATC TGGATGATC TTTCTATTTC GAAGGAAGCT  
 451 GTCATAGAAA TTACAGAAGT TCTCATTTGA AAGTTCCTCG AATGGTCTGC  
 501 TGAATTAAAA GCAAAATAGT AGGAACCTGT TGTGAAATG TCTATTTTAG  
 551 ATTCTTGGGC GAAACAATGC TTGAGCACAA TTTCTGAAAA TTACCGGTAT  
 601 CTGTCTCAG GTATCAATGC GTTCAGTTAC TTTACACGTC GCTATTAGAC  
 651 TACTCTGAA GAGTGGCTT COGGAGCATG GAGGTCTCGT TGTATTCTCT  
 701 CTGAGGGTCT ATCTCCAGAA GCTCAAAATC GTGTCTGTGA TATTAAGCGC  
 751 GTGTGAGATT ATATTAAATG GCATGATGTC AGTGTGGTTT TCCTTGAGGA  
 801 TACTCTGAAC CAGATGCGGT TGAAGAAAAT TGTTCCTCTC CTGAAGAAAA  
 851 GTCAATTAGT TCGTCTAGCT CAAAAACCAT TGTATAGTGA TATGTGGAC  
 901 GACAAATTAT TTAGCACCTT TAAACATAT GTCTGCCTTA TCACAGAAGA

951 ATTAGGAGGG GTGGCTCTTG AATGTCAAAG ATGA

The PSORT algorithm predicts inner membrane (0.168).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 76A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 76B) and for

FACS analysis.

The cp6624 protein was also identified in the 2D-PAGE experiment.

These experiments show that cp6624 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 77

10 The following *C.pneumoniae* protein (PID 4376728) was expressed <SEQ ID 153; cp6728>:

1 MKSSVSWLFF SSIPLFSSLS IVAAEVTLDS SNNSYDGSNG TTFVVFSTTD  
51 AAGGTTYSLL SDVSFQNGA LGIPLASGCF LEAGGDLTFQ GNCHALKPAF  
101 INAGSSAGTV ASTSAARDNL LFNDFSLRLSI ISCPILLSP TGQCALKSVG  
151 NLSLTNSQI LPTQNPSSDN GGVINTKMLF LSGTSQPAF SHNQAFTGKQ  
201 GNVVATSTI TIENSPGIVS FSNLAKGSG GALYSTNCS YDNPQVIFD  
251 GNSAMEAAQA QGGAICCTYT DKVTLKQNK NLSPTNCTT TYOGAISGLK  
301 VSI SAGPTL FQNSISGSSA QGGGGATNI ASAGFALISA TSGDITFNNN  
401 QTTNGSTSTR NAINIILDRK VTSIRAATGQ SIYPVDDITN POTAASDTLL  
451 NLNLADANSE LEYGGAIKVS GBKLSPTEKA IAAVNTSTIR QPAVLARGDL  
501 VLRDGVTVTF KDLTQSPGSR LMDGQTTLS AKEANLSIAG LAVNLSGLDG  
551 TNKAAIKTEA ADKNISLGGT TALIDTBGSF YENHMLKAS TYPLLELITA  
601 QANOTITLGA LSTPLIQEPE THYGVQNNQ LSWANATSEK IGSINWRTG  
651 YIPSPERKSN LPLNSLWGNF IDIRSTNQLT FTKSSGEPPE RELMLSGIAN  
701 KXNHGDTTGA SLYPHHTBGL PDIANFLWKG ATRAPWVLEF ISQIPLSFD  
751 AKPSYLVLTIN HMKTYYTDS IIRKGSWRND FCDLGLASLP FVIGVPLYLK  
801 EVFEPFKVKYQ IYAFQDFYE RHAEGRAPNK SELINVEIP IGVTFERDSKS  
851 EKGTVDLTLN YILDAYREND KCQTSILASD ANMWAYGTNL ARQSPSVRAA  
901 NHFQVNPME IPGQPAFEVR SSRNRYNXL GSKPCF\*

30 The cp6728 nucleotide sequence <SEQ ID 154> is:

1 ATGAAGTCCT CTGCTCTCTG GTTGCTCTTT TCTTCAATCC CGCTCTTTTC  
51 ATGCGCTCTCT ATAGTCGCGG CAGAGGTGAC CTTAGATAGC AGCAATAATA  
101 GCTATGATGG ATCTAACGGA ACTACCTTCA CGGTCTTTTC CACTACGGAC  
151 GCTCGTCAG GAACCTACCTA TTCTTACTTC TCGAGGTAT CTTTTCARAA  
201 TGCAGGGGCT TTAGGAATTC CCTTAGGCTC AGGATGCTTC CTAGAAGCGG  
251 GCGCGGATCT TACTTTCCAA GGAATACCA ATGCACTGAA GTTTCGATTT  
301 ATCAATGCGG GCTCTAGCGC TGGAACTGTA GCCATACCT CAGCAGCAGA  
351 TAAGAATCTT CTCTCTTAATG ATTTTCTTAG ACTCTCTATT ATCTCTGTCT  
401 CCTCTCTTCT TCTCTCTCTT ACTGGACAAT GTGCTTTAAA ATCTGTGGGG  
451 AATCTATCTC TAACGTGGCA TTCCCAAAAT ATATTACTTC AGAACTCTCT  
501 GTCAGATAAC GCGGTGTGTA TCAATACGAA AAACCTCTTA TTATCAGGGA  
551 CATCTCAGTT TCGGAGCTTT TCGGAAGAAC AAGCTTTCAC AGGGAAGCAA  
601 GCGCGGTGAG TTACGCTAC AGGAACATA ACTATCGAGA ACAGCCCTGG  
651 GATAGTTTTC TTCTCTCAAA ACCATGCGAA AGGATCTGCG GGTGCTCTGT  
701 ACAGCACTGA CAAGTGTTCG ATPACAGATA ACTTTCAAGT GATCTTTGAC  
751 GGCATAAGTG CTTGGGAAGC CGCTCAAGCT CAGGCGCGGG CTATTGTGTG  
801 CACTACGACA GATAAAACAG TGACTCTTAC TGGGAACAAA AACCTCTCTT  
851 TCACAAATAA TACAGCATTTG ACATATGGCG GAGCCTCTCT TGGACTCAAG  
901 GTCAGTATTT CCGCTGGAGG TCCTACTCTA TTTCAAAGTA ATACTCTCAG  
951 AAGTAGCGCC GGTACGAGG GAGGAGGAGC GATCAATATA GCATCTGCTG  
1001 GGGAACTCGC TCTCTCTCTT ACTCTTGAG ATATTACTCT CAATPACAAAC  
1051 CAGGTACCCA ACCGGAACGAC AAGTACAAAG AACGCAATTA ATATCTATTG  
1101 TACCGCTAAA CATCACATCG TACGAGCTGC TACGGGCGAA CTATCTATTG  
1151 TCTATGATCC CATCACAAAT CCAAGAACCG CAGCTCTCAT CGACACATTT  
1201 AACTTAAACT TAGCAGATGC GAACAGTGAG ATCGAGTATG GGGTCCGATG  
1251 TGTCTTTTCT GGAGAAAAGC TTTCCTCTAC AGAAAAGCA ATCGCTGCAA

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1301 ACGTCACCTC TACTATCCGA CAACCTGCAG TATTAGCGCG GGGAGATCTT  
 1351 GTACTTCGCT ATGGAGTCAC CGTAACCTTC AAGGATCTGA CTCAAAGTCC  
 1401 AGGATCCCGC ATCTTAATGAT ATGGGGGAGC TACACTTAGT GCTAAGAGGG  
 1451 CAAATCTTTC GTTAAATGGC TTAGCAGTAA ATCTCTCCTC TTTAGATGGA  
 5 1501 ACCAACAAAGG CAGCTTTTAA AACAGAAAGCT GCAGATAAAA ATATCAGCCT  
 1551 ATCGGGAACG ATTGCGCTTA TTGACACGGA AGGGTCACTT TATGAGAATC  
 1601 ATAACCTTAA AAGTGTAGT ACCTATCCTC TTCTTGAATC TACCACGCGA  
 1651 GGAGCCAAAG GAACGATTAAC TCTGGGAGCT CTTTCTACCC TGACTCTTCA  
 1701 AGAACCTGAA ACCCCTACTG GGTATCAAGG AAACCTGGAC TTGTCTTGGG  
 10 1751 CAAATGCAAC ATCTTCAAAA ATAGGAAGCA TCAACTGGAC CCGTACAGGA  
 1801 TACATTCCTA GTCCCTGAGAG AAAAAGTAAT CTCCCTCTAA ATAGCTTATG  
 1851 GGGAAACTTT ATAGATATAC GCTCGATCAA TCAGCTTATA GAAACCAAGT  
 1901 CCAGTGGGGA GCCTTTTGAG CGTGGAGCTAT GGCTTTCAGG AATTGCGAAT  
 1951 TTCTTCTATA GAGATTCTAT GCCACCOCG CATGGTTTCC GCCATATCAG  
 15 2001 CGGGGGTTAT GCATAGGGA TCACAGCAAC AACTCCTGCC GAGGATCAGC  
 2051 TTAATTTTTC CTCTGCGCAG CTCTTTGCTA GAGATCGCAA TCATATTACA  
 2101 GGTAAAGAAC ACGGAGATAC TTACGCTGCC TCTTTGTATT TCCACCATAC  
 2151 AGAAGGGCTC TTGCACATCG CCAATTTCTC CTGGGGAAAA GCAACCOGAG  
 20 2201 CTCCCTGGGT GCTCTCTGAG ATCTCCCAAG TCATTCCTTT TCGTTCGATC  
 2251 GCTAAATCTA GTTATCTCCA TACAGACAAC CACATGAAGA CATATTATAC  
 2301 CGATAACTCT ATCATCAAGG GTTCTTGGAG AAACGATGCC TTCTGTGACG  
 2351 ATCTTGGAGC TAGCCGTGCT TTTGTATTAT CCGTTCGGTA TCTTCTGAAA  
 2401 GAAGTCGAAC CTTTGTCTCA AGTACAGTAT ATCTATGCGC ATCAGCAAGA  
 2451 CTTCTACGAG CGTCATGCTG AAGGACGCGC TTTCAMTAAA AGCGAGCTTA  
 25 2501 TCACAGTAGA GATTCCTATA GCGCTCACCT TCGAAGAGAA CTCAAAATCA  
 2551 GAAAAGGGAA CTTACGATCT TACTCTTATG TATATGAAG ATGCTTACCG  
 2601 ACGCAATCCT AATGTCTCAA CTTCCTTAAT AGCTACGATG GCTAATGGA  
 2651 TGGCCTATGG TACCAACCTC GCACGACAAG GTTTTCTGCT TCGTGTGCGG  
 2701 AACCATTTCC AAGTGAACCC CCACATGGAA ATCTTCGGTC AATTGCTTTT  
 30 2751 TGAAGTACGA AGTCTTTCAC GAAATTTATA TACAAACCTA GGCTCTAAGT  
 2801 TTTGTTTCTA G

The PSORT algorithm predicts inner membrane (0.187).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 77A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 77B) and for FACS analysis.

The cp6728 protein was also identified in the 2D-PAGE experiment.

These experiments show that cp6728 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 78

The following *C.pneumoniae* protein (PID 4376847) was expressed <SEQ ID 155; cp6847>:

1 MPVMKILVRL CVVLLSLLPN VLFSSDLLRE EGKKRMDKL IEYHVDQAEV  
 51 STFDLBSRLS SYIQSFDPHK SYLSNQEVAV FLQSPETKKR LLKNYKAGHF  
 101 AIYRNINQLI HESILRARQW RNEWVKNPKE LVLEASSYQI SKQPMQWSKS  
 151 LDEVKQRQRA LLLSYLSLHL AGASSSRYEG KEEQLAALCL RQIENHENYV  
 201 LGINDHGIVAM DRDEEAYQPH IRUVKALJHS LDAHTAFYFK DRALAMRIQL  
 251 EKGMCIGIVV LKEDIDGVVV REIIPGGPAA KSGDLQGLDI IYRVGDKDIE  
 301 HLSPRGVLDL LRGGHGSTVV LDIHRGESDH TIALRREKIL LDRRRVDVSY  
 351 EPYGDGVIGK VTLHSFYEGE NOVVSSEQLR RAIQQLKERK LGLVLDIRE  
 401 NTGGFLSQAI KVSLGFMFWG VVVVSRYADG TMRCYRTVSP KKFYDGLAI  
 451 LVSKSSASAA EIVAQTLQDY GVALVVGDBQ TYGKGTIQHQ TITGDASDD  
 501 CFKVTVGKYY SFSGKSTQLQ GVKSDILIPS LYAEDRLGER FLEHPLPADC  
 551 CDNVLDHPLT DLDTQTRPWF GRYYLENLQK QETLWREMLP QLTNKSQRLL  
 601 SENSTNQAFI SKIKSSEKTD LSYGSNDLQL EESINILKDM ILLQCCRK\*

A predicted signal peptide is highlighted.

The cp6847 nucleotide sequence <SEQ ID 156> is:

1 ATGTTCTGTAA TGAAAAAAGCT TGTCGCTCTA TGCCTAGTTC TTCTTTCTTT  
 5 151 ACTTCCGAAT GTATTTATTT CTTCGGATCT TTACAGAGAA GAGGCGATCA  
 101 AAAAGATGAT GGACAAGCTG ATCGAGTATC ATGTTCGATGC TCAAGAGGTT  
 151 TCTACGGATA TACTCTCGCG TTCTTTATCT AGTTACATTC AATCTTTTGA  
 201 TCCTCATAAA TCTTATCTTT CAAAACCAAG GGTTCGACGT TTCTTCACAGT  
 251 CTCCGGAAAC AAGAAACGCT CTCTTAAAGA ATTATAAGAG AGGCAACTTT  
 301 GCTATTTATC GCACATATCA TCAATTAAAT CATGAGAGTA TTCTTCGTGC  
 351 CAGGCAGTGG AAGAACGAA GGGTTAAGAA TCCAAAGAG CTCTGTATTGG  
 401 AGGCATCTCT ATATCAGATA TCGAAGCAAC CTATGCAATG GAGCATACTC  
 10 451 TTAGACGAGG TGAAGCAGAG ACAACGCGCT CTACTCTTTT CCTATCTTTT  
 501 TTTACATCTT GCTGGAGCTT CTTCCTCTCG TTATGAGGGT AAGAAGAGC  
 551 AGCTTGTCTG TGTGTGTCTA CGTCAAAATCG AGAACCATGA GAATGTATAT  
 601 TTAGGTATCA ACGATCATGG TGTGTCTATG GATCCGGATG AAGAAGCCTA  
 651 CCAATTCAT ATCCGTGTGT TTAAGCTTT AGCTCATAGC TTAGATGCAC  
 15 701 ATACGGCGTA TTTCACTAAG GACGAAGCTT TGGCGATGCG AATCCAATCA  
 751 GAAAAAGGCA TGTGTGGAAT TGGTGTGTGT CTGAGGGAAG ATATTGATGG  
 801 AGTGTGTGTT AGAGAAATCA TTCTCTGGGG ACCTGCGGCT AATCTGGGG  
 851 ATCTCTAGCT TGGAGATATC ATCTATCGGG TGGATGGCAA GGATATCGAG  
 901 CATCTTCTTT TCCCGCGTGT TTAGAGTTGT TTACGTGGAG GTCATGGGCTC  
 951 TACTGTAGTC TTAGATATCC ATCGTGGGA GAGCGATCAT ACGATCGCTC  
 1001 TGAGAAGGGA GAAATCTCTT TTAGAAGACC GCTGCTGGA TGTTCCTTAT  
 1051 GAGCCTTATG GAGATGGTGT GATTGGGAAA GTTACGTTAC ATTCCTTTTAT  
 1101 TGAAGGAGAA AATCAGGTTT CTAGTGAACA AGATCTACGT CGAGCGATTC  
 1151 AGGGATTAAG GAGGAGAAAC CTCTCTGGAT TAGTTTGA TAATCGAGAA  
 25 1201 AATACGGGTG GATTTTTATC TCAAGCGATC AAGTTTCTGT GTTATTTTAT  
 1251 GACCAATGGC GTTGTGGTGT TATCTCGCTA TGCTGATGGT ACCATGAAGT  
 1301 GCTACCGCAC AGTATCTCCT AAAAAATCT ATGATGGTCC TTGTGCTATT  
 1351 TTAGTATCTA AAGGTTCGCG ATCAGCAGCG GAGATTGTAG CACAACTCT  
 1401 CCAAGATTAT GGAGTGTCTT TAGTTGTGTG AGATGAGCAG AACTATGGGA  
 30 1451 AGGGAACGAT TCAGCATCAA ACAATTACTG GAGATGCTTC TCAGGACGAT  
 1501 TGTTTTAAGG TTAATGTAGG GAATATTAT TCCCTTCTGT GGAAATCGAC  
 1551 TCAACTTCAG GGAGTAAAT CCGATATTTT AATTCTCTCT CTCTATGCTG  
 1601 AAGATCGTCT AGGAGAGCGT TTTCTAGAGC ATCCCTTACC TGCAGATTGC  
 1651 TGTGATAATG TACTTTCACA TCCTCTCAGC GACTTTGATA CTCACACAG  
 35 1701 TCTTGGTGT CAAAAATACT ATCTTCTCAA TCTACAAAG CAGAGAGCTC  
 1751 TTTGGAGAGA GATGCTACCT CAGCTTACGA ABAACAGTGA GCAAAGCTC  
 1801 TCTGAGAAAT CGAATTTTCA GGCATTTTGT TCGCAGATAA AATCATCTGA  
 1851 AAAAACGGAC CTATCTCTATG GTTCCAATGA TTTCAATTG GAAGAGTCGA  
 1901 TAAACATTTT GAAGGACATG ATTTTATATC AACAGTGTAG AAAATAA

40 The PSORT algorithm predicts periplasmic (0.932).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 78A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 78B) and for FACS analysis.

These experiments show that cp6847 is a surface-exposed and immunoinaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 79

The following *C. pneumoniae* protein (PID 4376969) was expressed <SEQ ID 157; cp6969>:

1 MRLFSLOTLY LFPSLALSSC CGYSILNSPY HLSSLGKSL QERIFIAPIK  
 51 EDPHGQLCSA LYELSKRSF ALSGRSSCAG YLAKVELLNG IDKNIGFTYA  
 101 PNKLGDKTHR HFIVSNEGR LLSAEVQLIN NDTQVILIDQ CVARESVDPE  
 151 FEPDLGTANA HEFALGQFEM HSEAKSARR ILSTRLAETI AQVYVYDF\*

A predicted signal peptide is highlighted.

The cp6969 nucleotide sequence <SEQ ID 158> is:

1 ATGAGATTGT TTTCTTTAGG CACGATTAT CTCTTTTATT CTCTAGCACT  
 51 TTCTGCTATG TGTGGTACT CTATTTTAAA CAGCCCGTAT CACTATCGT  
 101 CTTTAGGTAA GTCTTTATTA CAGGAAGAA TTTTCAATTG TCCCATAAA

151 GAAGATCCCTC ATGGTCAGCT CTGCTCAGCT CTAACCTATG AGCTTAGTAA  
 201 GCGTCTCTTT GCTATCTCTG GAAGAGCTTC TTGCGCAGCG TATACTCTTA  
 251 AAGTATGAGCT TCTGAATGGT ATTGACAGA ATATAGGTTT TACGTATGCC  
 301 CCAATTAAC TCGAGATAA GACTCACAGG CATTTTAPAG TCTCTAATGA  
 351 AGGACAGCTA TCACTATCTG CAAAAGTACA GCTTATCAAT AATGACACTC  
 401 AAGAAGTCTT TATAGACCAA TCTGTTGCTC GAGAGTCTGT AGACTTTGAC  
 451 TTTGAGCCTG ACTTAGGAAC AGCAAAAGCT CATGAATTGG CTTTAGGCCA  
 501 ATTTGAAATG CATAGTGAAG CCATAAAAG TGCTCGCGT ATACTATCTA  
 551 TAGCGCTAGC CGAGACGATT GCTCAACAGG TATACTATGA CCTTTTGTGA

10 The PSORT algorithm predicts inner membrane (0.126).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 79A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 79B) and for FACS analysis.

These experiments show that cp6969 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 80

The following *C.pneumoniae* protein (PID 4377109) was expressed <SEQ ID 159; cp7109>:

1 MKKTCQNYR SIGVVFSVVL FVLTTQPLFA GHFIDIGTSG LYSWARGVSG  
 51 DGRVVVQYEG GNAFKYVDGE KFLLEGLVPR SEALVFKAQY DGSVILIGSD  
 101 QDPSCRAVKW VNGALVDLGI FSEGMQSFAP GVSSDGKTIV GCLYSDDTET  
 151 NFVAKWDETG MVVLPNLPED RHSCAMDASE DGSVIVGDAM GSEBIKAVY  
 201 WKDGECHLLS NIPGAKRSSA HAVSKDGSFI VGEFISEENE VHAFVYHNGV  
 251 IKDIGTLGGD YSVATGVSRD GRVIVGHSTR TDGEYRAFVY VDGRMIDLGT  
 301 LGGSASFAPG VSDDGKRTVG KFEDELGECH AFYILD\*

25 A predicted signal peptide is highlighted.

The cp7109 nucleotide sequence <SEQ ID 160> is:

1 ATGAAAAAGA CATGTTGCCA AAATTACAGA TCGATAGGCG TTGTGTTCTC  
 51 TGTGGTACTT TTCGTTCTTA CAACACAGAC GCTGTTTGCA GGACATTTTA  
 101 TTGATATTGG AACTCTCGGA TTATATTCTT GGGCTCGAGG TGTATCTGGA  
 151 GATGGCCGCG TTGCTGAGG TTATGAAGGT GGCAATGCAT TTAATATATG  
 201 TGATGGTGAG AATTTCTGT TAGAAGGTTT GGTCCGAGGA TCGAGGCCCT  
 251 TGGTATTAA AGCTTCTTAT GATGCGCTCT TAATTATAGG AATCTCGGAT  
 301 CAAGAATCGT CTGCGCGCG TGTGAAGTGG GTAAACGGTG CACTGTTTGA  
 351 TCTTGGAAAT TTTTCTGAGG GAATGCAATC TTTTGACAGG GGTGTTTCCA  
 401 GTGATGGAAA GACGATGTGA GGTGCGCTAT ATAGTATAGA TACAGAGACA  
 451 AACTTTGCTG TGAAGTGGGA TGAACAGGGA ATGGTTGTTC TCCCTAACTT  
 501 ACCAGAAAGT CGACATCTTT CGGCTTGGGA TGCCCTCGAA GATGGCTCTG  
 551 TGATTTGAGG GGACGCCATC GGTAGCGAGG AAATGCGCAA GGCAGGTGAC  
 601 TGGAAAGGAC GTGAACAACA TCTGCTTTCT AATATCCGAG GAGCTAAAG  
 651 ATCGTTCAGCA CATGCAGTTT CTAAGATGAG ATCTTTTATC GTAGCGGAGT  
 701 TCACTCAGTA AGAAAAATGAA GTTCATGCC TTTGTTATCA CAACGGTGT  
 751 ATCAAAGATA TCGGAGCTTT AGGAGCGAGT TACTCTGTAG CACCTGGAGT  
 801 TTCTAGGAGT GGTAAAGTCA TCGTGGGTCA TTCTACAAGA ACAGATGGTG  
 851 AAATACCGTGC ATTTAAATAT GTGAGATGGAA GAATGATAGA TTTGGGAGCT  
 901 TTAGGAGGTT CAGCATCTTT TGCTTTTGGT GTTCTGAGC ATGGCAAAAC  
 951 AATCGTAGGA AATTTGAAA CAGAGCTAGG AGAATGTCAT GCCTTTATCT  
 1001 ACCTTGATGA TTAG

The PSORT algorithm predicts outer membrane (0.887).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 80A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 80B) and for FACS analysis.

These experiments show that cp7109 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 81

The following *C.pneumoniae* protein (PID 4377110) was expressed <SEQ ID 161; cp7110>:

```

1  MAAIKQILRS MLQSSSLWV LFSLYSLGY CVYITDKPED DPHSSSAVKWN
51 DHMKITLSR LSNKKASAKA VSGTGATTVG FIKDINSTRGY AUKWNYWGTFK
101 ELPTSSSWVK SKATGISSDG SIIRAGIVENE LQSFATVWK NEMNYLLPST
151 WAVQSKAYGI SSDGSIVVGS AKDAMSRTFA VKWTGHEAGV LEVQWAVKSV
201 ANSVSANGSI IVGSVQDASG ILYAVKNEGN TITHLGLQGG YSLAKAVSN
251 NGRVIVGRSE TTYGEVHAFK HKNGVMSDLG TLGGSYSAK GVSKATKVIV
301 GMSTTANGKL HAFKIVGGRM IDLGEYSWKE ACANAVSIDG ELIVGVQSE*
```

A predicted signal peptide is highlighted.

The cp7110 nucleotide sequence <SEQ ID 162> is:

```

1  ATGGCAGCTA TAAACAAAT TTACGTTCT ATGCTATCTC AGAGTAGCTT
51 ATGGATGGTC CTATTTTCAT TATATCTCTC ATCTGGGTAT TGCTATGTAA
101 TTACAGACAA ACCAGAAGAT GACTTCCATT CTTCATCCGC AGTAAATGG
151 GATCATTTGGG GAAAGACAAC TCCTCAAGA TTATCAAAAT AAAAGCCCTC
201 TGCNAAAGCT GTTTCAGGAA CTGGTGCTAC AACTGTCGGC TTTATAAAG
251 ACACTTGGTC TCGAACATAC GCAGTAAAGT GGAATATTG GGGGACCAA
301 GAACCTCCCTA CAGCTCATG GGTAAAAAAA TCAAAGCAA CAGGAATCTC
351 CTCTGATGGG TCTATAATCG CGGGGATTGT CGAGAAATGAG CTTTCTCAA
401 GTTTCGCAGT CACATGAGAA AACAAATGAA TGTATTGCT CCCTCCACA
451 TGGGCGATGC AATCTAAGAC GTATGGAATT TCTTCTGATG GCCTCTGTTAT
501 TGTAGGAGGT GCTAAGGATG CTTGCTCGCG AACTTTCGCT GTGAAGTGA
551 CGGGACACGA GGCTCAGGTG TTACCAAGTAG GCTGGGCTGT CAAATCTGTA
601 GCGAATTCCTG TATCTGCCAA TGGATCTATA ATGTAGGGT CIGTACAAGA
651 CGCCTCTGGA ATTCTTTATG CTGTAAAGTG GGAAGGAAAC ACTATTACAC
701 ATCTAGGAAC TATTAGGAGC TATTCTGCCA TTGCAAAAGC TGTATCCAAT
751 AATGGCAAGG TCATTGTAGG GAGATCGGAA ACATATTATG GAGAGGTCCA
801 TGCTTTCTGT CATAAGAAATG GCGTCANGTC AGACCTCGGC ACCCTCGGAG
851 GATCTTTATTC TGCAGCTAAG GAGTCTCTG CAACCTGGAAA AGTTATTGTC
901 GGTATGTCCA CAACAGCAAA TGGGAAATG CATGCCCTTA AATATGTGCG
951 TGGAAAGAAAT ATCGACTTAG GAGAGTATAG CTGGAAAGAA GCCCTGTGCAA
1001 ACGCTGTTTC TATTGATGGA GAAATTATTG TTGGAGTCCA ATCAGAAATA
```

35 The PSORT algorithm predicts outer membrane (0.827).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 81A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 81B) and for FACS analysis.

40 These experiments show that cp7110 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Figure 191 shows a schematic representation of the structural relationships between of cp7105, cp7106, cp7107, cp7108, cp7109 and cp7110, each of which is identified herein. These six proteins may be grouped in a new family of related outer membrane-associated proteins. These proteins have a repeat structure in common (*cf.* the pmp family).

### 45 Example 82

The following *C.pneumoniae* protein (PID 4377127) was expressed <SEQ ID 163; cp7127>:

```

1  MVFFRNSLKH LVALSGMLCC SSGVALTIAE KNASLEHSGR GADDYEGMAS
```

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|     |             |            |            |            |            |
|-----|-------------|------------|------------|------------|------------|
| 51  | FNAMREYSL   | QLSKLYEAR  | KLRASGTEDE | ALWKDLIRRI | GEVRYGLREI |
| 101 | RELWAAEIRE  | KGGNLEDYAL | WNHPETITYN | LVIDPGETDS | IVLIPQEIGA |
| 151 | IKIATLSKFV  | VPRKSFEDCL | TQLSLRLIG  | VRQVNSWIK  | LPMARKGCS  |
| 201 | VAGVFSSRKD  | LEALPETAYI | GFVLNSMVA  | HTNQVILKFK | INPETHVDV  |
| 251 | TAGRVWIFGS  | AGVAGELLKI | YNFVQSSIR  | QBYRVIPLIK | IDPGEMISL  |
| 301 | NAAPTEDELTK | DVSESLGGR  | VVFLQYGRS  | LFLSGTALV  | QQAULTIREL |
| 351 | EGELENPTK   | IVPFWYKIN  | DPCELAALLS | QVHWTSGBN  | KASVGAADGC |
| 401 | GSQNLASIQI  | DTTVSSBAKD | GSVKGNFIA  | LSKTOTLINV | VKEVILPRIQ |
| 451 | MLLKLIDVFK  | KWVIRFLVLF | ERKLALRQKS | GLMLRLRQGS | VCKKGSPSV  |
| 501 | SWAGSTGLE   | PLFGSTGSS  | IVPYDILAYQ | FLMAQSDVRI | NASPSVVTMM |
| 551 | QTPARIAVVD  | EMSTAVSSDK | DKAQVNRACV | GINIKIMFVI | NVGEEDGKSY |
| 601 | ITLFTDITFD  | ITGRNHDPR  | DVIRINNTMK | VRIADGETVI | IGGLRCQMS  |
| 651 | DSHDGIFPLG  | DTPIGKLFG  | MSSTDSBLTE | MFVPTTETKL | EMPVQCQERK |
| 701 | BEALLSSRPQ  | HRSEYQALA  | ASEAARAHA  | KKLEMFASG  | VSLSQVBRQE |
| 751 | YDC*        |            |            |            |            |

A predicted signal peptide is highlighted.

The cp7127 nucleotide sequence &lt;SEQ ID 164&gt; is:

|      |             |            |             |             |             |
|------|-------------|------------|-------------|-------------|-------------|
| 1    | ATGGTITTTT  | TCCGTAATTC | TTTACTGCAT  | TTAGTTGCC   | TATCCGGAAT  |
| 51   | GCTCTGTGTT  | TCCTTCGGAG | TGGCTTTAAAC | GATAGCCGAG  | AAGATGGCTT  |
| 101  | CTTTAGAGCA  | CTCGGAGAGA | GGAGCAGACG  | ATTATGAGGG  | GATGGCTTCG  |
| 151  | TTTAAATGCCA | ATATGAGGGA | GTATAGCCTT  | CAGCTAGATG  | AGTTGTATGA  |
| 201  | GGAAAGCAGA  | AAGCTACGCG | CTTCTGGAAC  | TGAGGATGAA  | GCTCTGTGGA  |
| 251  | AGGACTTAAT  | TCGACGGATT | GGTGAGGTGC  | GAGGCTATCT  | TCGAGAGATC  |
| 301  | GAGGAGCTTT  | GGGCTGCAGA | AAITCGTAG   | AAAGGGGGCA  | ATCTCGAGGA  |
| 351  | CTACGCCCTC  | TGGAATCACC | CAGAGACTAC  | GATTTACCAAT | CTTGTACC    |
| 401  | ATTACGGAAC  | CGAAGACTCT | ATTATTTTGA  | TTCCTCAAG   | AATCGGAGCG  |
| 451  | ATTAAATTCG  | CAACCTTATC | GAAATTTGTA  | GTTCCTAAG   | AGTCTTTCTGA |
| 501  | AGACTGTCTC  | ACTCAGATCC | TATCTCGCTT  | AGTATTTGGC  | GTGCGTCAGG  |
| 551  | TCAATTCTTG  | GATTAAAGAA | CTTTATATGA  | TGCGTAAGGA  | GGGCTCAGT   |
| 601  | GTTCGCTGAG  | TTTTTTCTCT | CAGAAAGATC  | TTAGAGGGCG  | TCCCAAGAAC  |
| 651  | AGCCTATATT  | GGTTTGTAT  | TGAAITCGAA  | CGTAGATCG   | CATACCAATC  |
| 701  | AACATGTCTT  | AAAAAAGTCT | ATTAAACCTG  | AAACAACGCA  | TGTAGATGTG  |
| 751  | ATTGCAGGAC  | GGTGTGGAT  | TTTTGGTTCT  | CGGGGGGAAG  | TCGGCGAGCT  |
| 801  | TCTGAAAGAT  | TATAATTTTG | TGCACTCGGA  | GAGCATACGT  | CAAGAGTATC  |
| 851  | GGGTGATTCC  | CTTAACTAAG | ATCGATCCAG  | GGGAGATGAT  | TTTCACTCTC  |
| 901  | AACGCAGCAT  | TTCTCGAGGA | TCTGACTAAA  | GATGTAGTGT  | AAAGATCTTT  |
| 951  | AGGCCTTCTG  | GTAGTTCTTT | TACAGTATCA  | AGGGCGTCTG  | TTGTTTTAA   |
| 1001 | GTGGAACCGC  | GGGCTTAGTG | CAGCAAGCGC  | TGACTCTCAT  | TCGAGAGCTT  |
| 1051 | GAAGAAGGGA  | TTGAGAACCC | TACGGATAAA  | ACAGTATTAT  | GGTATACGTT  |
| 1101 | CAAGCACTCC  | GATCCCCAAG | AGTTGGCGCG  | ATTGCTTCCC  | CAAGTCCATG  |
| 1151 | ATGTCPTCTC  | TGGCGAGAAT | AAGGCGAGTG  | TCGGAGCTGC  | AGATGGATGT  |
| 1201 | GGGTGCAAT   | TAAATGCCTC | GATCCAAATT  | GATACTACAG  | TAAATCTCTC  |
| 1251 | TGCAGAAAGT  | GGCTCAGTGA | AGTACGGAAA  | CTTCACTCGC  | GATCTAAGA   |
| 1301 | CAGGAACCTC  | GATTATGGTG | GTGAGAGAA   | AAGTCTCTCC  | ACGTATTCAG  |
| 1351 | ATGCTACTTA  | AGAAACTAGA | TGTCCCTAAA  | AAGATGCTCC  | GTATCGAGGT  |
| 1401 | GCTGTATTAT  | GAAAGAAAAT | TGGCACATGA  | GCAGAAATCT  | GGTTAAATCT  |
| 1451 | TTCTACGCTC  | TGGTGAGGAA | GTPTGTAAAA  | AAGGCTGACG  | TCTCTCTGTG  |
| 1501 | TCTTGGGCCG  | GGGGTACTGG | CATATAGAAA  | TTTTTTATTA  | AAGGAAGTAC  |
| 1551 | GGGATCTTCG  | ATAGTTCTTG | TTTATGATCT  | CGCTCTTCAA  | TTTTTAAATGG |
| 1601 | CTCAAGAGGA  | CGTTTCGGAT | AATGCGAGTC  | CTTCTGTAGT  | TACTATGAAC  |
| 1651 | CAAAACCCAG  | CACGGATTTC | TGTGTGTGAT  | GAAATGTCAA  | TAGCGGTGTC  |
| 1701 | TTCAAGATAA  | GATAAAGCGC | AATACATTCG  | TGCGCAGTAC  | GGTATCATGA  |
| 1751 | TAAAAATGCT  | CCCCGTAAAT | AATGTGGGAG  | AGGAAGAAGG  | AAAAAGTTAC  |
| 1801 | ATTACTTTAG  | AGACAGACAT | CACCTTTGAT  | ACTACGGAAA  | AAAAATCATGA |
| 1851 | TGATCTGTCT  | GATGTATCAA | GGCGTAATAT  | TACTATTAAG  | TGTGCGCAITG |
| 1901 | CTACGAGAGA  | GACTGTGATT | ATTGGAAGTT  | TGCGTGTCAA  | ACAGATGTCA  |
| 1951 | GATTCCTATG  | ATGGCATTCC | TTTTCTTGGG  | GACATCTCTG  | GTATAGGGAA  |
| 2001 | GTTATTCTGA  | ATGAGTTCCA | CATCAGACAG  | TCTCAGGAG   | ATGTTTGTAT  |
| 2051 | TTATCACCTC  | GAAGATCTTA | GAAATCCTTG  | TAGAGCAACA  | GAAGACGTAA  |
| 2101 | GAAGAAGCTT  | TACTCTCTTC | CGCCCTTGA   | GAGAGAGAAG  | AATACTATCA  |
| 2151 | GGCTTTAGCA  | GCTAGTGAGG | CTCAGACAG   | AGCAAGATAG  | AAAAAATTAG  |
| 2201 | AGATGTTCCT  | GGCATCAGGA | GTATCTTTAT  | CTCAGGTAGA  | GAGGCAAGAA  |
| 2251 | TACGATGGCT  | GCTAG      |             |             |             |

The PSORT algorithm predicts periplasmic (0.920).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 82A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 82B) and for FACS analysis.

These experiments show that cp7127 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 83

The following *C.pneumoniae* protein (PID 4377133) was expressed <SEQ ID 165; cp7133>:

```

1  MQPFIPTLLC LTSLVSLVAF DAANARKRCA CAQTIERGEN FFSIKRSACA
51 EIEYQEKSRH ASALERISKD KGVTPKQTA KVAATKKQRY RLLQVPFSRP
101 PNNISRYNLYA LLSPEPPCYS DTASWYAIFP RLLRRAYVDI QNVPPGSEYA
151 IANALISNKG EILERGAQLG PDVITLTLP EBAEAIYFYM LKGSNSQSLS
201 INFLHYEEKS LGHCKLNLIF MDPLLRLAVL DHPDAYRETS LLRDGIWEAV
251 KRQEHAIQEH QQAALBELFK TRTDFRIELR DKMQLLSRY DILPLLNKKM
301 FDYTLGSGAD YLFLVDPDTK AISRRCPSK SYKL

```

A predicted signal peptide is highlighted.

The cp7133 nucleotide sequence <SEQ ID 166> is:

```

1  ATGCAACCTT TTATCTTAC TTTACTGTGC TTGACATCTT TGGTTCTTT
51 AGTCGCCITT GATGCTGCGA ATGCTCGTAA ACOTGTGCGC TGTGCTCAAA
101 CTATAGAACG TGGAGAGAAC TTCTTTTCCA TAAAACGCTC TCGTGTGTCT
151 GAATCGAAT ATCAAGAAAA ATCTCGCCAC GCCTCAGCAA TTGAAGAAT
201 CTCAAAAGAT AAAGGCAAGG TCACCTCCAA GCAGATTCGG AAGTAGCTA
251 CTAAGAAAAA GCAAGATATC CGTTATATGC AGTTCTCTTT TTCAAGGCGT
301 CCGAATAACT CAAGGTATAA CCTCTATGCT TTGCTTAGTG AACCTCCGGA
351 ATGCTATAGC GATACAGCAT CATGGTATGC TATTTTATAT CGGTACTCTC
401 GACGCTGCTA TGTAGACAGC GGAAATGTAC CTCCTGGATC TGAGTAGGCC
451 ATCGCTAATG CTTTGATAGT TAACAAACAA GAGATTTTAG AGAGGGGAGC
501 GCAGCTTGGG CCGGATGTTA TTGAACTCT AACATTGCCT GAGGAACAG
551 CCGAGATTTT TTATATAATG CTCAAAGGGT CGTCAAACTC TCAGTCGCTA
601 CTGAATTTTC TGCATTATGA AGAGAAAGC TTAGGCCACT GTAAGCTAAA
651 TCTGATCTTC ATGGATCCCG TACTGTTAGA AGCTGTTCTA GATCATCCCG
701 ATGCTTATAG GGAACGTCG CTCCTGCGCG ATGGCATTTC GGAAGCGGTG
751 AAGCGTCAAG AACATGCCAT CCAAGAACAT GGCCAGGCAG CTGCTTTGGA
801 GCTTTTAAA ACACGCAACG ACTTCGCGCT GGAGCTCGGA GATAAGATGC
851 AGTTACTTCT AAGTCGATAC GATTTCCTCC CCTTATTAAT TAAAAAATG
901 TTCGACTACA CCTTAGGAAG TGCCGGAGAT TACTTATTTT TGGTAGACCC
951 AGATACTAAG GCAATTTCTC GATGTCGCTG CCTTCAGAG AGTATTAAAT
1001 TATAA

```

The PSORT algorithm predicts outer membrane (0.92).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 83A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 83B) and for FACS analysis.

These experiments show that cp7133 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 84

The following *C.pneumoniae* protein (PID 4377222) was expressed <SEQ ID 167; cp7222>:

```

1  MNRRDMVITA VVVNAILLYA LFVTSKRIGV KDYDEGFRNF ASSKVQAVV
51 SEERVKIEPV VAEVSPRPIA KEFLAAQFIE SKPFIIVITFP VVVVSTPEV

```



```

101 PTVAVPPQPV RBTVKEEQAP YATVVVVKGD FLERIARANH TTVAKLMQIN
151 DLTTTLQKIG QVTKVPTSQD VSNKPTQQT TAMPENYIV QBGDSFWTLA
201 LKNHRLDDL LKNDLDEYK ARRLKPGDQL RIR*

```

A predicted signal peptide is highlighted.

- 5 The cp7222 nucleotide sequence <SEQ ID 168> is:

```

1 ATGAATCGTA GAGACATGGT AATACAGCT GTCGTAGTGA ATGCTATATT
51 GCTTGTGGCT CTTTTCGTCA CATCAAGCG TATTGGCTC AAGGACTATG
101 ACGAGGGATT CCGTAATTTT GCTTCTAGCA AGGTTACACA AGCAGTAGTT
151 TCAGAAAGAAA AAGTCATAGA AAGCCGTGA GTCCGAGAA GTCCTAGCCG
201 TCCTATCGCT AAGAGACTC TAGCTGCACA GTTTATTGAA AGTAAGCCGG
251 TTAATTGTAAC CACACCACC GTGCTGTTG TTAGCGAAG CCCAGAAGTG
301 CCTACTGTGG CAGTCCCGCT TCAGCTGTT CGTGAGACAG TAAAGAGGGA
351 ACAAGCTCCT TATGCTACTG TTGTAGTGA AAAAGGAGAT TTCTCGAAC
401 GCATTGCGAG AGCAATCAT ACTACCGTTG CAAATTTGAT GCAGATCAAT
451 GATCTTACCA CCACCAACT TAAATTTGTT CAGGTATCAT AAGTCCCTAC
501 GTCTCAAGAT GTCAGCAAC AAAAATCTC TCAACACAG ACCGCAAAAC
551 CTGAAAATTA TTATATCGTC CAAGAAGGGG ATAGCCCGTG GACAATAGCA
601 TTGCGTAACC ATATCTGATT GGATGATTGT CTAATAATGA ATGATCTCGA
651 TGAATATAAA GCCCGCGCC TTAAGCCTGG AGATCAGTTG CGCATACGTT
701 GA

```

The PSORT algorithm predicts periplasmic (0.935).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 84A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 84B) and for FACS analysis.

- 25 These experiments show that cp7222 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 85

The following *C. pneumoniae* protein (PID 4377225) was expressed <SEQ ID 169; ep7225>:

```

1 MKGPQYHFI GIGGIGMSAL AHILLDRGYE VSGSDLYBSY TIESLKAKGA
51 RCFSGHDSH VPHDAVVVYS SSIAPDNVEY LTAIQRSSRL LHRAELLSQL
101 MBGYSEILVS GSHGKTGTS LIRAI PQEAG KDPSTAGGL AANCLNGYSG
151 SSKIFVARAD BSGDSLKHYT PRAVITNID NEHLNMYAGN LDNLVQVQID
201 FSRKVTDLNK VFYNGDCPLL KGNVQGISY YSEPCQLHIV SYNGKAWQSH
251 PSFTTFIGQY QITLNLPLGQ HNAANAAAC GVALTFGIDI NTRKALKPK
301 SGVHRLERK NIESPLFLE DYAHHPVEVA HTLRSDVDAV GLRRVIAIPQ
351 PHRFSLREC LQTFPKAFQE ADEVLITDVI SAGESPRESI ILSDLAEQIR
401 KSSYVHCYV PHGDIVDYLR NYIRIHVDCV SLGAGNIYTI GEALKDFNPK
451 KLSIGLVCGG KSCHEHISLL SAQHVSRYIS PEFYDVSYP IINRGQLWRTG
501 KDFPHLIEET QGDSPLSSEI ASALAKVDCL FVPLHGPFGE DGTIQGFPEI
551 LKGPYAGPSL SLAATAMDKL LTKRIASAVG VPVVPYQPLN LCFWKRNPPEL
601 CIGNLITFVS FPMIVKTAHL GSSIGIFLVR DKEELQEKIS EAFLYDITDV
651 VESRLGSRB LEVSCIGHSS SWCMAGPNE RCGASGFIDY QKRYGFDGID
701 CAKISFDLQL QDESLDVRE LAERVYRAMQ GKGSARIDFF LDEBGNWLS
751 EVNPLFGMTA ASPFLQAFVH AGWTQSQIVD HFILDLHFK DKQQTIBQAF
801 TREQDLVRK*

```

The cp7225 nucleotide sequence <SEQ ID 170> is:

```

1 ATGAAGGGAA CTCTCAGTA TCATTATAT GGTATCGGTG GTATAGGAAT
51 GAGCGCTTTA GCTCATATTT TGCTTGATCG TGCTATGAG GTCTCTGGAA
101 GCGACTTATA TGAAGAGCTAT ACGATCGAAA GCTCTGAAAG TAAAGGTGCG
151 AGGTGTTTCT CAGGCAATGA TTCTCTCCAT GTTCTCATG ATGCGCTCGT
201 TGTATTATAG CTAAGTATAG CCCTCGATAA TTAGAGATAT CTTACCGCTA
251 TTCAAGATC ATCAGCTCTT CTTCATAGAG CAGAGCTCTT GAGTCAGCTT
301 ATGAGAGGTT ATGAAGCAT TCTGGTTTCA GGAAGCATG GGAAGACAGG
351 GACCTCATCT CTAATTCGAG CGATTTTCCA GGAAGCTCAG AAGATCCCT

```

|    |      |            |            |             |             |            |
|----|------|------------|------------|-------------|-------------|------------|
|    | 401  | CCTATGCTAT | TGGAGGACTC | GCTGCAAACT  | GCCTGAATGG  | GTATTCGGA  |
|    | 451  | TCATCGAAAA | TCTTCGTTGC | CGAAGCCGAT  | GAAGTGAATG  | GGTCTTTAAA |
|    | 501  | GCACTACACT | CCCGTGCAG  | TAGTCRTTAC  | AAATATAGAT  | AATGAACATT |
| 5  | 551  | TGAATAATTA | CGCTGGGAAT | CTGTATTAAC  | TGGTTCAGGT  | AATCCAGGAC |
|    | 601  | TTCTCTAGAA | AAGTAACAGA | TCTCAATTAAG | GTATTCATTA  | ACGGGGATTG |
|    | 651  | TCCTATTTTG | AAGGAAATG  | TCCAAAGGAT  | TTCTTATGGA  | TATTCACCA  |
|    | 701  | AATGTCAATT | GCATATCGTT | TCCTATTAATC | AAAAGGCATG  | GCATATCCAC |
|    | 751  | TTTTCTTTTA | CTTTTITTAG | CCAGGAGTAT  | CAAGACATAT  | ACGTCAATCT |
| 10 | 801  | CCCTGGAACA | CATAACGCTG | CAATGTCAGC  | AGCAGCCCTG  | GGAGTTGGCT |
|    | 851  | TTACCTTTGG | CATAGACATA | AACTHCACTT  | GAAGAAGCTC  | CAAAAHAATC |
|    | 901  | TCGGGAGTTC | ATCGACGTCT | AGAAAGAAAA  | AATATATCCG  | AAAGCTTTCT |
|    | 951  | TTTCTTAGAA | GATTATGCTC | ATCATCTCTG  | AGAGGTTGCA  | CATACCTGTC |
|    | 1001 | GCTCTGTGCG | TGATGCTGTG | GGTTTGGCAG  | GAGTCAATGCG | AATTTTTCAA |
|    | 1051 | CCACATCGAT | TCTCTCTGTT | AGAAAGTGTG  | TTACAAACCT  | TCCCAAAAGC |
| 15 | 1101 | TTTCCAGAA  | GCTCATGAAG | TGATACTTAC  | AGATGTCTAT  | AGTGCCGAGC |
|    | 1151 | AAAGTCCTAG | AGAGTCTATC | ATTCTTTCCG  | ACCTTCGGGA  | ACAGATPTCG |
|    | 1201 | AAAGTCTTCT | ATGTCCTATG | TGTGTATGTT  | CCCTATGGAG  | ACATCGTAGA |
|    | 1251 | TTATCTACGA | AATCAATTC  | GCATTCATGA  | TGTCTGTGTT  | TCTCTAGGAG |
| 20 | 1301 | CTGGAATAT  | CTATACTATT | GGAGGGCTGT  | TAAAAGACTTT | TAACTCTAAA |
|    | 1351 | AAATTAATCA | TAGACTCTGT | CTGTGGAGGG  | AAATCTTTGCG | AACAGATAT  |
|    | 1401 | TTCTCTACTT | TCTGCTCAAC | ATGTCCTCAA  | ATATATTTCT  | CCTGAATPCT |
|    | 1451 | ATGATCTGAG | TACTTTCATC | ATAATCTGTC  | AGGCTTTATG  | GAGAACAAGA |
|    | 1501 | AAGGATTTTC | CTCATCTTAT | TGAAGAGACT  | CAAGGGGATTT | CGCCACTTTT |
|    | 1551 | TTCTGAAATC | GCTTCAGCTT | TAGCAAAAGT  | CGACTGTGTT  | TTTCCCGTGC |
| 25 | 1601 | TCCATGGCCC | ATTTGGAGAG | GATGTACAGA  | TCCAGGGATT  | TTTTGAAATC |
|    | 1651 | TTAGGAAAC  | CTATGGCCGG | ACCCTCACTA  | TCTTTGTCAG  | CAACTGCAAT |
|    | 1701 | GGATAAGCTG | TTAACAAAC  | GAATTGCTCT  | AGCAGTGGGT  | GTCTCTGTAG |
|    | 1751 | TCCTCTACCA | ACCTTTAAAT | CTCTGTCTTC  | GGAAACGCAT  | TCCGAACATA |
|    | 1801 | TGATATTCGA | ATCTTATAGA | GACATTTTCT  | TTCCCTATGA  | TTGTAAHAAC |
| 30 | 1851 | TGCACATTGG | GGATCTAGTA | TTGGGATATT  | TTTAGTCCGT  | GATAAAGAGG |
|    | 1901 | AATTACAAGA | AAAGATCTCA | GAAGCATTTT  | TATATGACAC  | GGATGTGTTT |
|    | 1951 | GTGGAGGAAA | GTGCTGTAGG | GTCTCGTAA   | ATCGAAGTGT  | CCTGTATCGG |
|    | 2001 | CCATTCCTCT | AGCTGGTATT | GTATGGCAGG  | GCCTAATGAA  | CGCTGTGGTG |
|    | 2051 | CTAGTGGGTT | TATTGATTAT | CAAGAGAAAT  | ATGGATTGGA  | TGGCATAGAT |
| 35 | 2101 | TGGCGAAGA  | TCTCTTTTGA | TTTACAGCTC  | TCAACAGAAAT | CTTTAGATTG |
|    | 2151 | TGTTAGAGAA | CTTGCAGAGC | GTGTCACCCG  | AGCAATGCAA  | GGAAAGGTTG |
|    | 2201 | CAGCTCGAAT | AGATTTTFTT | TTGGATGAAG  | AGGGGAATTA  | TTGGTTGTCA |
|    | 2251 | GAGGTCAATC | CTATTCCAGG | AATGACAGCA  | GCTAGCCCAT  | TTTTCAAGCG |
|    | 2301 | TTTTGTCTAC | GCAGGATGGA | CGCAAGACCA  | AATGTATAGAT | CACTTTATTA |
| 40 | 2351 | TAGATGCTCT | ACATAAGTTT | GATAAGCAGC  | AGACTATGGA  | ACAGGCATTC |
|    | 2401 | ACTAAGAAC  | AAGATTTAGT | TAAAGATAA   |             |            |

The PSORT algorithm predicts inner membrane (0.16).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 85A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 85B) and for FACS analysis.

These experiments show that cp7225 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 86

The following *C.pneumoniae* protein (PID 4377248) was expressed <SEQ ID 171; cp7248>:

|    |     |            |            |            |             |            |
|----|-----|------------|------------|------------|-------------|------------|
| 50 | 1   | MKFWLQGCAP | VGCILLTLPC | CAARRRASGE | NLQOTREPIAA | ANLQWESYAE |
|    | 51  | ALHEHSQDHR | PICLFTTGSD | WCMWCIRMQD | QLQSSEFEKH  | FAGVHLHMYE |
|    | 101 | VDFPQKNHPQ | EEQRQKNQEL | KAQYKVTGFP | ELVFIDAEKG  | QLARMGFPEP |
|    | 151 | GGAAVSVKVK | SALKLR*    |            |             |            |

A predicted signal peptide is highlighted.

55 The cp7248 nucleotide sequence <SEQ ID 172> is:

1 ATGAAATTTT GGTTCGCAAGG ATGTGCTTTT GTCGGTTGTC TGCTATTGAC

51 TTTACCTTGT TGTGCTGCAC GAAGACGTGC TTCTGGAGAA AATTTGCAAC  
 101 AAACCTCGTC TATAGCAGCT GCAATCTTAC AATGGGAGAG CTATGCAGAA  
 151 GCTCTTGAAC ATTCTAACA AGATCACAAA CCTATTGTTC TTGTCTTTAC  
 201 AGGATCAGAC TGTGTATGT GTGCGGAGAA AATGCAAGAC CAGATTTTGC  
 251 AAAGCTCTGA GTTTAAGCAI TTGCGGGTG TGCATCTGCA TATGTTTGAA  
 301 GTTGATTTC CCCAAAGAA TCATCAACCT GAAGAGCAGC GCCAAAAAA  
 351 TCAGAACTG AAGCTCAAT ATAAAGTTAC AGGATTCCTC GAATCTGCTT  
 401 TCATAGATGC AGAAGAAAA CAGCTTGCTC GCATGGGATT TGAGCCTGTT  
 451 GGTGGAGCTG CTACGTAAAG CAGGTGGAAG TCTGCTCTTA AACTACGTGA  
 501 A

The PSORT algorithm predicts periplasmic (0.932).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 86A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 86B) and for FACS analysis.

15 The ep7248 protein was also identified in the 2D-PAGE experiment.

These experiments show that cp7248 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 87

The following *C. pneumoniae* protein (PID 4377249) was expressed <SEQ ID 173; cp7249>:

20 1 MIPSPPTINF RDDTILETDP KPFLIMFSSK KTEIASERRK AHPTLFKVLG  
 51 TIMNIVKFI ILLFLFLAL LNLWKKTCQF FILPSSIIISQ SMKTAVAIR  
 101 RMTFLSHIKQ LLSLKEISAA DRVVIQVDDL VVD SLAIKIP HALPERWILY  
 151 SQGMSGLMEN LDRGDSSLL QLAATGSSNL LVNYPGIMS SKGBAKRENL  
 201 VSYQACVRY LRDEETGFKK NQITAFSYSL LVGWSAALD REVPDSDPT  
 251 SWIVKIDRGF RSLADVAMQ CKELASALIK LVGWSIDSVK PSERLCRPI  
 301 FLYNSNHQDE LISDGLPERE NCVATPPELE FVKTSQTKI FIPERDLHL  
 351 NPLSPNVVDR LAARVISNYLD SENRKSQQFD \*

The cp7249 nucleotide sequence <SEQ ID 174> is:

30 1 ATGATCCCAT CCCCTACCCC AATAAACTTT CGTGATGATA CGATTCTAGA  
 51 GACGGATCCA AAGCCGCTCTT TAATCATGTT CTCTTCAAAA AAAACAGAGA  
 101 TAGCTCTCTGA AAGACGGAAG GCCCATCCCA CCTATTPTAA AGTTCTAGGA  
 151 ACGATTCTGGA ATATTGTGAA GTTTATTATC TCAATCATTC TGTCTCTCC  
 201 CTTAGCGTTA TTGTGGGTAC TCAAGAAAC CTGTACGTTT TTCAATTCTCC  
 251 CATCTCTTAT CATATCTCAG AGCATGTCAA AAACAGCTGT GGCAATTCCG  
 301 CGAATGAOCT TTCTGTCCCA TATTAAACAA CTCTAAGCC TTAAGGAAT  
 351 CTCAGCTGCC GATCGTGTGG TTATACAATA TGACGATTGT GTGGTTGATA  
 401 GCTTAGCTAT AAAGATACCT CATGCTCTC CCCACAGGTG GATTCTTTAT  
 451 TCTCAAGGAA ACTCTGGATT GATGGAACAC CTGTTCGATC GGGGCGATTTC  
 501 CTCTCTACAC CAGCTAGCCA AAGCAACCG CTGGAATCTT CTGTGTTCAC  
 40 551 ACTATCCTGG AATTATGTCC AGCAAAAGAG AAGCGAAAG AGAAATCTGT  
 601 GTTAATCOT ATCAGGCATG CGTACGCTAC CTACGAGATG AAGAGACAGG  
 651 TCTTAAAGCC AATCAAAATCA TAGCTTTTGG ATACTCTTTC GGAATAGTG  
 701 TCCAAGCTGC TGCTCTAGAT CGTGAGGTCA CTGATGCGAC TGATGGAAT  
 751 TCATGGATTG TTGTAAAGA TCGGGGCCCT CGCTCTCTAG CAGATGTCCG  
 45 801 GAATCAAAAT TGTAAAGCCA TAGCTTCCGC GATTATATAAA CTCGTGGTT  
 851 GGAACATAGA CTCTGTGAAA CCTAGOGAAA GATTTCGTTG TCCCGAAATT  
 901 TTCATTTACA ACTCTAATCA TGATCAAGAA CTCATTAGCG ACGGCTCTCT  
 951 CGAAAGAGAA AATTGCGTAG CAACACCTTT TTAGAGACTT CTGGAAGTAA  
 1001 AAACTCCGGG GACTAAAAAT COTATACCCG AAAGGGATCT TCTCCATCTA  
 50 1051 AATCCTCTCA GTCCAAATGT AGTAGACAGA TTACGACAGT TGATCTCTAA  
 1101 TTAATTAGAT TCTGAAAAAC GAAAGTCTCA GCAACCTGAT TAA

The PSORT algorithm predicts inner membrane (0.571).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 87A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 87B) and for FACS analysis.

These experiments show that cp7249 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 88

The following *C.pneumoniae* protein (PID 4377261) was expressed <SEQ ID 175; cp7261>:

```

1  MLPISILLFY  VIIGCLSAYI  ADKKRRNVIG  WFFAGAFFGF  IGLVVLILLP
51  SRRNALEKPK  NDPPFNSDLF  DDLKKSLAGN  DELIPSSGLQ  ELVIDTERWF
101 YLNKDRKENV  PISFEELVVL  LKGGTYPEBI  WVKKGKMKDV  QRVKDVPSLQ
151 QALKEASK*
```

The cp7261 nucleotide sequence <SEQ ID 176> is:

```

1  ATGCTCCCTA  TTTCGATTIT  ATTATITTA  GTGATTCTAG  GTTGTCTATC
15  TGCTCFACATA  GCAGATAAGA  AAAAACGMAA  TGTATTATGCG  TGGTTTTTGG
101 CAGGAGCATT  TTTTGGATT  ATTGGTCTAG  TGTCTCTTCT  TCTTCTTCTT
151 TCTCGTCGAA  ACGCTTTAGA  AAAGCCACAA  AACGATCCTT  TTGATAACTC
201 CGATCTTTT  GATGATTTCGA  AAAAAGTTT  AGCAGGTAA  GACGAGATC
251 CCTCATCGGG  AGATCTTCAA  GAAATCGTTA  TCGATACAGA  GAAGTGGTTT
301 TATTTAAATA  AAGATAGAGA  AAACGTAGGT  CCGATATCTT  TTGAGGAGTT
351 GGTCTGACTT  TTAAGGGGAA  AAACGTATCC  AGAAGAAATT  TGGGTATGGA
401 AAAAGGGAAT  GAAAGATTGG  CAACGATGGA  AGGATGTTC  ATCACTACAA
451 CAGGCTTTGA  AAGAAGCATC  AAAATAA
```

The PSORT algorithm predicts inner membrane (0.848).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 88A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 88B) and for FACS analysis.

These experiments show that cp7261 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 89

The following *C.pneumoniae* protein (PID 4377305) was expressed <SEQ ID 177; cp7305>:

```

1  MEVVSFHPAV  RTSTQHRVMA  ALDAMFTLGG  HRLKVSVLDS  CNSGWAYQEL
51  VSLSTTEKVL  KLLSYLLVPI  VIALLIIRCL  LHSNPRIDVE  KERWLKIREL
101 GIDIBCKRLP  SYVNVQVSF  IWFEKDKSKR  PRIDVDYHEL  HSKDWVFFPI
151 VFQKIPKTSR  FSYVNSQKET  RKRDYVRNML  DHVIGYLTGE  GGHWLVISK
35  201 TSYQSATSLD  PERVLQYCLT  DNQELQGEVQ  RLNEESAFK  SSGDKVLLS
251 HVSDIICQCN  WPKFLEVIQS  PAFIEELVEE  VSKNLNLDPL  CLEKANTLDQ
301 ELRNSLLRAV  VHHGSEGVDI  KKVAGGLIY  TBAIQLQIFP  SRS*
```

The cp7305 nucleotide sequence <SEQ ID 178> is:

```

1  ATGGAAGTTT  ATAGTTTCA  CCCTGCGGTA  AGGACTTCGT  TTCAGCACCG
40  51  TGTAATGGCA  GCACTAGATG  CTTGGTITTT  TCTAGGAGGG  CACCGTTTAA
101 AAGTAGTTTC  TCTAGATAGT  TGTAACTCAG  GTTGGCGGTA  TCAGAACTT
151 GTGTCATTAT  CAACGACAGA  AAAAGTCTTG  AAACACTACT  CTTACCTACT
201 CGTACCGATT  GTCATAATAG  CTCGTGTAAT  TCGTTGTCTT  TTACATAGCA
251 ATTTTAGGAT  AGACGTAGAG  AAGGAACGTT  GGTTAATAAT  AAGGGAGTTA
45  301 GGAATTGATA  TAGAAAGCTG  CAAACTCCCC  AGTTCCTTATG  TAAACAGAGT
351 TTCTCTCGTT  ATTTGGTTTG  AAAAAGATAA  ATCCAAACGG  CCACGTATTG
401 ATGTAGATT  TCATACGCTA  CATAGCAAG  ACTGGGTAGT  TTTCCCTATC
```

|      |             |             |            |             |             |
|------|-------------|-------------|------------|-------------|-------------|
| 451  | GTTTTCAGAA  | AAATTCCAAA  | GACCTCGCGT | TTCAGTTATT  | GGTCTCACA   |
| 501  | AAAAGAAACA  | AGGAGAGGG   | ATTATGTGAG | AAATATGCTG  | GACCAAGTCA  |
| 551  | TGGGTTATCT  | AACGTCAGAA  | GGTGGGGAGT | GGTTGCAAGTA | TATATCGAAA  |
| 601  | ACCTCTTATC  | AAAGCGCTAC  | TTCTTSGAT  | CCTGAAGAG   | TTCTTCAATA  |
| 651  | TGCTCTAACT  | GATACCCAGG  | AGCTCCAGGG | AGAAGTGCAA  | CGTTTGCCTTA |
| 701  | ATGAGGAGAG  | TGCGACCAAA  | AGCTCTGGGG | ATAAGGAAGT  | TTTCTTAAGT  |
| 751  | CATGATATCT  | ACATTTATTTG | CAAGTGTGAG | TGGCCAAAGT  | TTCTTGAAGT  |
| 801  | TATACAATCT  | CGCGCTTTTA  | TTGAAGAATT | AGTGAAGAA   | GTGAGTGGTA  |
| 851  | AACCTTAATTT | AGATTTTTTTA | TGCTTGAAGA | AGGCTAATAC  | ATTAGATCAG  |
| 901  | GAGTTGAGAA  | ACAGTCTTCT  | AAGAGCAATC | GTACACCAAG  | GTTCTGAAGG  |
| 951  | AGTTGATATT  | AAGAAGTTTG  | GTCCGCGGCT | CATTATTTAT  | ACGGAAGCTA  |
| 1001 | TTCAATTACA  | GATTCCCTTC  | TCAAGGAGTT | AA          |             |

The PSORT algorithm predicts inner membrane (0.508).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 89A) and also as a double GST/his fusion. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 89B) and for FACS analysis.

These experiments show that cp7305 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 90

The following *C.pneumoniae* protein (PID 4377347) was expressed <SEQ ID 179; cp7347>:

|     |             |            |            |            |            |
|-----|-------------|------------|------------|------------|------------|
| 1   | MKKGLQIAIV  | PGLLFTSSVA | GFSGDLTKDN | AYQDLNVIEH | LISLKYAPLP |
| 51  | WKELLFQWDL  | SQQTQQAQLQ | LVLEKKPTTN | YQKVLNHYV  | RLNDYHAGI  |
| 101 | TFYRTESAYI  | PVYLKLSEDD | HVFVVVDQTS | QGDYILGDEI | LEVDMGIRE  |
| 151 | ATLESIRFRG  | SATDYSAAVR | SLTSSRAAFG | DAVPSGIAML | KLRFPGLIR  |
| 201 | STPVRWRVTP  | EHIGDFSLVA | PLIPEHKPQL | PTQSCVLFRS | GVNSQSSSSS |
| 251 | LFSSYVMPYF  | WEELRVQNKQ | RFDNSHHIGS | RNGFLPTTGP | ILWEGDKGYP |
| 301 | RSYIFKAKDS  | QGNPHRIGFL | RISSYVWTDL | EGLEEDHKDS | PWELFGEIID |
| 351 | HLKETDALJI  | IDQTHNPGGS | VFLYLSLLSM | LTDHPLDTPK | HRMIFTQDEV |
| 401 | SSALHWQDL   | EDVTFDEQAV | AVLGETMEGY | CMDMHVASL  | QNFQSQVLSS |
| 451 | WVSGDINLSK  | PMPLIGFAQV | RPHPKHQYTK | PLFMLIDEDD | FSCGDLAPAI |
| 501 | LKDNNGRATLI | GKPTAGAGGF | VFQVTFPNRS | GIKGLSLTGS | LAVRKDGFEI |
| 551 | ENLGVAPHID  | LGFTSRDLQT | SRTDYVEAV  | KTIVLTSISE | NAKRSBQTS  |
| 601 | PQETPEVIRV  | SYPTTTSAS* |            |            |            |

A predicted signal peptide is highlighted.

The cp7347 nucleotide sequence <SEQ ID 180> is:

|      |             |            |            |            |            |
|------|-------------|------------|------------|------------|------------|
| 1    | ATGAAAAAAG  | GGAATTAGS  | AGCCATAGIT | TTTGGCCTTC | TATTTACAAG |
| 51   | TAGTGTGCTT  | GGTTTCTCTA | AGGATTGAC  | TAAAGACAAC | GCTTATCAAG |
| 101  | ATTTAAATGT  | CATAGAGCAT | TAAATATCGT | TAAATATGCT | TCTTTTACCA |
| 151  | TGGAAGGAAC  | TATTATTGGG | TTGGGATTTA | TCTCAGCAAA | CACAGCAAGC |
| 201  | TGCGTTGCAA  | CTGGCTTAG  | AGAAGAAACC | AACAACCAAC | TACTGTCAGA |
| 251  | AGGTACTCTC  | TAACCTAGTG | AGATCATTAA | ACGATTATCA | TGCAAGGATT |
| 301  | ACGTTTATCT  | GTACTGAAGG | TGCTATATTC | CCTTACGTAT | TGAAGTTAAG |
| 351  | TGAAGATGGT  | CATGCTTTTG | TAGTCGACGT | ACAGACTAGC | CAAGGGGATA |
| 401  | TTTACTTAGG  | GGATGAUAAT | CTTGAAGTAG | ATTGCAATGG | GATTCGTGAG |
| 451  | GCTATCGAAA  | CGCTTCGCTT | TGACGAGGGG | AGTGCACACG | ACTATTCTGC |
| 501  | TGCACTTCGT  | TCTCTACAT  | CGCGTTCGCG | CGCTTTTGGA | GATGCGGTTC |
| 551  | CTTCAAGGAT  | TGCCATGTG  | AAACTTCGCC | GACCAAGTGG | TTTATCCGCT |
| 601  | TGCAACCCGG  | TCCGTTGGCG | TTATATCTCA | GAGCATATCG | GAGATTTTTC |
| 651  | TTTAGTTGCT  | CCTTGAATTC | CTGAACATAA | ACCTCAATTA | CCTACACAAA |
| 701  | GTGTGTGCTT  | ATTCCGTTCC | GGGTAATAAT | CACAGTCTTC | TAGTAGCTCT |
| 751  | TATTTCAGTT  | CCTACATGCT | GCCTTATTTT | TGGGAAGTCA | TCTGGGTTCA |
| 801  | AAATAGACAG  | CGTTTGTGCA | GTAATCTACA | TATAGGAGAG | CGTAATGGAT |
| 851  | TTTACCTTAT  | GTGTTGCTCT | ATTCTTTGGG | ACAAGACAAA | GGGGCCCTAT |
| 901  | CGTTACCTATA | TCTTTAAAGC | AAAGATATCT | CAGGCAATTC | CCCATCGCAT |
| 951  | AGGATTTTAA  | AGAAATTTCT | CTTAGTTTGG | GACTGATTTA | GAGGACCTTG |
| 1001 | AAGAGGATCA  | TAGAGGATAG | CCTTGGAGAG | TCTTTGGAGA | GATCATCGAT |

|      |            |            |            |             |             |
|------|------------|------------|------------|-------------|-------------|
| 1051 | CATTTGGAAA | AAGAGACTGA | TGCTTTGATT | ATTGATCAGA  | CCCATAAATCC |
| 1101 | TGGAGGCGAT | GTATTCTATC | TCATTCGTT  | ACTATCTATG  | TTAACAGATC  |
| 1151 | ATCCTTTAGA | TACTCTTAAA | CATGAAATGA | TTTTCACTCA  | GGATGAATTC  |
| 1201 | AGCTCGGCTT | TGACTTGCCA | AGATCTACTA | GAAAGTGCTC  | TCACAGATGA  |
| 1251 | CGAGGCACTT | GCCCTGCTAG | GCGAAACTAT | GGAAGGATAT  | TGCATGGATC  |
| 1301 | TGCATGCTGT | AGCCTCTCTT | CAAAACCTCT | CTCAGAGTGT  | CCCTTCTTCC  |
| 1351 | TGGGTTTCAG | GATGATATTA | CCCTTCAAAA | CCATATGCCCT | TGCTAGGATT  |
| 1401 | TGCACAGGTT | CGACCTCATC | CTAAACATCA | ATATACATAA  | CCCTTGTGTTA |
| 1451 | TGTTGATAGA | CGAGGATGAC | TTCTCTTGTC | GAGATTATGC  | GCGTGCAGTT  |
| 1501 | TTGAAGGATA | ATGGCGCGCG | TACTCTCAT  | GGAAGGCCAA  | CAGCAGGAGC  |
| 1551 | TGGAGGTTT  | GTATTCGAAC | TCACCTTCCC | TAAACCGTCT  | GGAAATTAAG  |
| 1601 | GTCTTCTCTT | AACAGGATCT | TTAGCTGTGA | GGAAGATGG   | TGAGTTTNTT  |
| 1651 | GAAAACTTAG | GAGTGGCTCC | TCATATTGAT | TTAGGATTTA  | CCTCCAGGGA  |
| 1701 | TTTGCAAACT | TCCAGGTTTA | CTGATTACGT | TGAGGCAGTG  | AAAACATATG  |
| 1751 | TTTTTAACCT | TTTGTCTGAG | AACGCTAAGA | AGAGTGAGAA  | GCAGACTTCT  |
| 1801 | CCGCAAGAGA | CGCTGAAGT  | TATTCGAGTC | TCCTATCCCA  | CAACGACTTC  |
| 1851 | TGCTTCGTAA |            |            |             |             |

The PSORT algorithm predicts periplasmic space (0.2497).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 90A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 90B) and for FACS analysis.

These experiments show that cp7347 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 91

The following *C. pneumoniae* protein (PID 43737353) was expressed <SEQ ID 181; cp7353>:

|     |            |            |            |             |            |
|-----|------------|------------|------------|-------------|------------|
| 1   | MNMPVPSAVP | SANITLKEDS | STVSTASGIL | KIATGEVLVS  | CTALEGSSST |
| 51  | DALISLALGQ | LILATQQLL  | LQSTNVHQLL | FLPPEVVELE  | IQVVDLLVQL |
| 101 | EHAETITSEP | QETQTQSRSE | QTLFQQSSSK | QSALSFRSLK  | FEISDSKQQQ |
| 151 | ALQTPKDSAV | RKHSEAFSPE | TQARASLSQA | SSSSQSRSLP  | QESAPERTLL |
| 201 | EQQKASSFSP | LSQFSAEKOK | EALTTSRSH  | LYKERDQDRQ  | QREQHDRKHD |
| 251 | QEEDAESKKK | KKRKLGVREA | VAEPEGRNLD | LAALIPSDQM  | RPPAETSKK  |
| 301 | ETTFKKKLPS | PMSVFSRFP  | SKNPLSVGSS | IHGPIQTQPKV | ENVFLRFMKL |
| 351 | MARILQAEAE | EANELYMRVK | QRTDDVDTLT | VLISSKINNEK | KDIDWSENEE |
| 401 | MKALLNRAKE | IGVTIDKEYK | TWTEERRL   | KENVQMRKEN  | MEKITQMERT |
| 451 | DMQRHLQEIS | QCHQARSNVL | KLLKELMDTF | IYNLRP*     |            |

The cp7353 nucleotide sequence <SEQ ID 182> is:

|      |            |            |            |            |            |
|------|------------|------------|------------|------------|------------|
| 1    | ATGAATATGC | CTGTTCCCTC | TGCAAGTCCC | TCTGCRAATA | TAACTCTAAA |
| 51   | AGAAGACAGC | TCAACAGTTT | CCACAGCCTC | TGGAATATTA | AAGACTGCAG |
| 101  | CAGTGGAAGT | CTTAGTCTCT | TGTACAGCGC | TAGAAGGAAG | CTCTTCTACA |
| 151  | GATGCTTTAA | TAGCTTAGCT | TTTAGACAAA | ATCATTCTTG | CGACCCAAAC |
| 201  | AGAAGTCTCT | TTACAAAGCA | CAAAATGTTC | TCAACTCTCT | TTCTCCCTCC |
| 251  | CTGAAGTTGT | AGATTAGAAA | ATCCAAGTTG | TGAGCTTGCT | AGTGCAATTG |
| 301  | GAACATGCAG | AGACATACCA | AAGTGAACCA | CAGAAACAC  | AAAGCGAAG  |
| 351  | TAGGAGTGAG | CAGACCTCTC | CTCAACAAG  | CAGCAGTAAA | CAATCTGCTC |
| 401  | TCTCCCCACG | CTCTTTAAAA | CTGAAATTT  | CTGATTCTAA | ACAACAGCAA |
| 451  | GCTCTTCAAA | CACCAAAAGA | CTCTGCTGTA | AGAAAACACA | GCGAAGCACC |
| 501  | GTCACTGAG  | ACACAAGCTC | GCGCTTCTCT | ATCTCAGGCA | AGCTCAAGTT |
| 551  | CTCAGAGATC | CTTACTCTCG | CAAGAAAGTG | CGCCAGAAAG | AAACATATTA |
| 601  | GAACAACAAA | AGCAAGCTC  | CTTCTCTCTC | CTATCCAGAT | TCTCTGAGA  |
| 651  | GAAACAAA   | GAGGCCCTGA | CGACTCAAA  | ATCTCATGAA | CTCTATAAAG |
| 701  | AACCGCATCA | AGATCGCCAA | CAAGAGAGAG | AGCAGACAGC | AAAGCCAGAT |
| 751  | CAGGAAGGAG | ACGCTGATCT | TAAAAGAGAA | AAAGAGAAAC | GTGGTCTCGG |
| 801  | TGTAGAGGCA | GTCGCTGAGG | AACCCGAGAG | AAATCTAGAT | ATTGCCGCTT |
| 851  | TAACTCTCTC | AGATCAAAAT | CGACTCTCTG | CTGAAGAAAC | TTCTTAAAAA |
| 901  | GAAACGACAT | TCAAAAAGAA | GCTACCTTCT | CCAATGTCTG | TGTTTAGACG |
| 951  | ATTCACTCCT | AGTAGAATCA | CGTTATCTGT | AGGCTCTTCA | ATACACGGGC |
| 1001 | CTATACAAAC | TCCAAAAGTA | GAAAATGTGT | TCTTAGGTTT | CATGAGCTCT |

1051 ATGGCAAGAA TCCTAGGCCA AGCCGAAGCC GAAGCTAATG AACTCTACAT  
 1101 GCGAGTCAAA CAACGTACCG ATGATGTAGA CACACTCACA GTCTTATCT  
 1151 CTAAGATCAA TAATGAAAAG AAAGACATTG ATTGGAOTGA AAATGAAGAG  
 1201 ATGAAAGCTC TTTTAAATCG AGCTAAGAG ATTGAGTCA CTATAGACAA  
 1251 AGAAAATAT ACITGGACAG AAGAGGAAAA AAGACTTCTA AAAGAGAATG  
 1301 TCCAAATCG CAAAGAGAAT ATGGAGAAAA TCACTCAAAAT GSAAGGACG  
 1351 GACATGCAAA GGCACCTCCA AGAGATTCTT CAATGTCAAT AAGCGCGCTC  
 1401 TAATGTATTG AAGTATTATG AAGAACTTAT GGACACCTTC ATTACAAACC  
 1451 TACGCCCTTA A

10 The PSORT algorithm predicts cytoplasm (0.1308).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 91A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 91B) and for FACS analysis.

15 These experiments show that cp7353 is a surface-exposed and immunoreaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 92

The following *C.pneumoniae* protein (PID 4377408) was expressed <SEQ ID 183; cp7408>:

20 1 MLKIQKRMK VSVVITVGA I VGFNSADAA PKKKIPIQ I LYSFTKVSSY  
 51 LKNEASTIF CVDVDRGLLQ HRYLGSFGWQ ETRRRQLFKS LENQSYGNER  
 101 LGEETLAIDI FRNKECLESE IPEQMEAILA NSSALVLGIS SFGITGIPAT  
 151 LHSLLRQNL S FQRSLASES FLKIDSAPS DASVFKYGLV FRGETAIVDA  
 201 LSQFLAQDL SPKKIIFLGE DPEVVQAVGS ACIGWGMNFI GLVVYPAQES  
 251 LFSYVHPYST ATELQEAQGL QVLSDEVAQL TLNALPKHN\*

The cp7408 nucleotide sequence <SEQ ID 184> is:

25 1 ATGTTGAAAA TCCAGAAAAA AAGAATGTGT GTCAGCGTAG TCATCACGGT  
 51 AGGCGCCATA GTGGGGTTTT TCAATCTCTG AGACGCAGCA CCAAGAAAAA  
 101 AGAAGATCCC TATACAGATT CTCTACTCCT TTAATAAGT CTCTCTCTAT  
 151 TTAATAAACG AAGACGCAAG TACTATATT TCGCTCGATG TGGATCGTGG  
 201 ACTTCTCCAG CATCGGTATT TAGGTAGTCC AGGATGGCAG GAAACCAAGC  
 251 GTCCGCGAGT ATTTAAATCC TTAGAAAAAT AATCATACGG CAACGAACGT  
 301 TTAGAGAGAAG AAACCTTTGC TATTGATATT TTACAGAAC AAGAGTGCTT  
 351 GGAGAGCAG AGATCCAGAGC AGATGGAAGC TATCTTTGCA AATCTCTCGG  
 401 CCTTGGTCTT AGGCATCTCT TCTTTTGGGA TCACAGAAAT TCCTGCGACT  
 451 TTGCATAGTT TGCTTCGACA GAATCTATCT TTCCAAAAAC GCTCTATAGC  
 35 51 ATCGGAGAGC TTCTTTTAA AGATCGATAG TGCCCTCTCA GATGCTCTGT  
 551 TTTTATTAA AGGCGTGCTT TTCCGCGGAG AGACTCGCAT CGTGGATGCG  
 601 TTAAGCCAAT TATTTCGCCA GCTCGATCTT TCTCTTAAAA AAATATCTTT  
 651 TCTAGGAGAA GACCTTGAGG TCGTTCAAGC TGTGGGTCT GCTTGTATAG  
 701 GTTGGGCGAT GAACTTTATA GGCTTGGTAT ACTATCTGTC TCAAGAAAGC  
 40 751 CTTTTCCTT ATGTCATCT TACTCTACA GCAACGAGC TCAAGAAAGC  
 801 ACAGGGTTTA CAAGTAATTT CAGATGAAGT CGCAGAGCTT ACTTTAAACG  
 851 CTCTCCGAA AATGAATTAA

The PSORT algorithm predicts inner membrane (0.123).

45 The protein was expressed in *E.coli* and purified as a his-tag product (Figure 92A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 92B) and for FACS analysis.

These experiments show that cp7408 is a surface-exposed and immunoreaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

**Example 93**

The following *C.pneumoniae* protein (PID 4376424) was expressed <SEQ ID 185; cp6424>:

```

1 MMHNIIVLSE EPGRSAFLGR TAFFPNKYPI AQGVGIPST IGLNFTIWWC
51 FVFYRAATPQ SDHPDGCQFT LLERLKEGGA GFFYCDLRES NTTGFTLFFE
101 GSNKGVLLNH LFRIDE*

```

The cp6424 nucleotide sequence <SEQ ID 186> is:

```

1 ATGATGCACA ATATTGTTGT TCTTAGTGAG GAACCTGGAC GAAGCGCTTT
51 TCTTGCTAGG ACGGCATTTT TCCTTAATAA GTATCCAATA GCTCAGGCTG
101 GTGTGGAAT ACCACTTACA ATAGCAATC TCTTACTACT ATGTAATCTG
10 151 TTCTATTFTT ATAGAGCTGC AACTCCACA TCTGATCATC CTGACGGATG
201 TGGCTTTATT CTACTAGAAA GGCTTAAGGA GCTCGGTGCA GGGTCTCTTT
251 ATTGTGATCT TCGTGAOTCC AATACCACGT GCTTTACTCT TTTTITTGAA
301 GCTCCCAATA AAGGTGTGTT AAGAATCAC TTGTTTATTA GAGATGAGTA
351 A

```

15 The PSORT algorithm predicts cytoplasm (0.2502).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 93A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figure 93B) and for FACS analyses (Figure 93C; GST-fusion).

20 These experiments show that cp6424 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

**Example 94**

The following *C.pneumoniae* protein (PID 4376449) was expressed <SEQ ID 187; cp6449>:

```

1 VASETPSQI LHAQREVDA YFNQADCHPA RANQILEAKK ICLLDVYHTN
25 51 HYSVFTFCVD NYPNLRPTFV SSKNNEMNGL SNPLDNVLVE AMVRRTHARN
101 LLAACKIRNI EVPRVVLDDL RSGILISKLE LRQPFQSLT EDFVNHSTNQ
151 EEARVHKHV LLISLILLCK QAVLESFQEK KRSS*

```

The cp6449 nucleotide sequence <SEQ ID 188> is:

```

1 GTGGCGCTCTG AAACGTATCC TTCTCAGATA TTGCACGCTC AGAGGGAAGT
51 ACGTGATGCC TATTTTAATC AAGCGGATTG CCACTCGTGT CGGGCTAATC
30 101 AGATTCTCGA GGCTAAGAAA ATCTGTTTAT TAGATGTTTA TCATACTAAT
151 CATTAATCCG TATTACTTCT TTGTGTAGAT AATTATCCGA ATCTCCGCTT
201 TACATTGTGA TCTTCAAAAA ACAATGAGAT GAAATGCCTA TCTAATCCTC
251 TAGATAATGT TCTTTAGAG GCTATGGTAC GTAGAACACA TGCAAGAAAC
301 CTACTGTCAG CGTGTAAAAA TCGAAATATG GAGOTCCAAA GGGTGTGTGG
35 351 GCTTGACCTA AGATCTGGGA TACTCATTTT GAAACTAGAA TTGAAGCAAC
401 CTCAGTTCOA AAGTTTAAAC GAAGACTTCG TAAATCATTC CACAAATCAC
451 GAAGAAGCTC GCGTCCATCA AAAGCATGTG TTGCTAATTT CTTTAATTTT
501 ACTTTGCAAG CAGGCCGTTT TGGAAATCAT CCAGGAAAAA AAGCGATCTC
551 CTAA

```

40 The PSORT algorithm predicts inner membrane (0.2084).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 94A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figure 94B) and for FACS analyses (Figure 94C; GST-fusion).

45 These experiments show that cp6449 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.



**Example 95**

The following *C.pneumoniae* protein (PID 4376495) was expressed <SEQ ID 189; cp6495>:

MRELNAFELTQPEEYENRWLMPCLKCRFCRTQHAQVSWYRCHVEASLYEKNCFLLTYDDKHLFQYGSILVKLHLQLFLKRLR  
LRKMISPHKIRYFECGAYGFKLQRPHYHLLS

- 5 The cp6495 nucleotide sequence <SEQ ID 190> is:

TTGCGAGAATTAAATGCTTTTGAATTAACCTCAACCTGAAGAGTATCGAAACCGTTGGGTTTGTATGCCCTGTCTTAAGTGT  
CGTTTGTGTAAGACCGCAACATGCAAAAGTCTGGTCTTATCGTTGGTCCATGGAAGCTTCTTTGTATGAGAAAAATTTGTTT  
CTTACTTTTGAATCTATGATGATAAGCAATTTACCTCAGTATGGTTCGTTGGTAAAGCTGCATTTACAGCTGTCTCTTAAGAGA  
TTAAGAAAGATGATTTCTCTCATATAAAATTCGTTATTTTGAATGGTGGTATGGAACCAATACCAAGACCTCATTTAT  
CATCTACTTTTATCATGA

10

The PSORT algorithm predicts cytoplasmic (0.280).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 95A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 95B) and for FACS analysis (Figure 95C).

15

These experiments show that cp6495 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

**Example 96**

The following *C.pneumoniae* protein (PID 4376506) was expressed <SEQ ID 191; cp6506>:

20

1 MRRFLFLILS SLPLVAFSAD NPTILEEKQS PLSRVSIIFA LPGVTPVPSFD  
51 GNCPIWFPSH SKKTLGGRI YYSQDSFGKY FVVSALMPNK VSSAVVACNM  
101 ILKHRVDLIL IIGSCYRSQ DSRFSGVLVS KGYINYDADV RPFPERFEIP  
151 DIKKSVPFAS EVHREAILRG GEEFISTHKQ EIEELKTHG YLKSTTKTEH  
201 TMEGLVATG ESFAMSRNYP LSLQKLYPEI HGFDVSQAV SQVCYEYSIP  
251 CLGVNILLPH PLESRNEDW KHLQSEASKI YMDTLKLSVL KELCSSH\*

25

The cp6506 nucleotide sequence <SEQ ID 192> is:

30

1 ATGCGTCGTT TTCTGTTTCT TATTCTTAGC TCTCTCCCT TGGTCGCAAT  
51 CTCTGCTGAT AATTTCACCTA TTCTAGAGAG AAAACAGAGT CCTTTAAGTC  
101 GTGTAAAGAT TATTTTGCTT TTACCTGGGG TTACTCCCGT TTCTTTTGAAT  
151 GGTAAATGTC CTATTCCTCTG GTTTCTCTCA AGTAAAGAA CTTAGAGGGG  
201 ACAGAGAATT TATTACTCTG GCGACTCCTT TGGGAATAT TTTGTAGTTT  
251 CTGCTCTCTG CCTATAAATA GTTTCTTCAG CTGTTGTGGC TTGTAATATG  
301 ATTCTTAAAC ATCAGAGTGA TCTTATTCTA ATTATAGGCT CGTGTTACTC  
351 TAGGTCCTCAA GATAGCCGTT TTGGCAGGCT CTTAGTTTCT AAAGCTACA  
401 TTAATTATGA TGCAGATGTG AGGCTTTTCT TTGAAGAATT TGAGATTCCA  
351 GACATTAATA AGAGTGTGTT TGCAACAGT GAGGTCTACG GGGAGGCAAT  
501 TCTTCGTGGA GCGCAAGAGT TTTATTCTAC CCAATAACAA GAATTCGAAG  
551 AGCTTTTGAA GACTCATGGG TATTGTGAAA CAACAACCAA AACGGAGCAC  
601 ACCTTAATGG AAGGTTTGGT TGCTACAGGC GAGTCTTTCG CGATGTCGCG  
651 AAACATATTCT TTCTCCTTAC AAAAATGTGA TCCAGAGATT CATGTTTGTG  
401 701 ATAGTGTCTG CCGCGCTGTT TCTCAGGATT GCTATGAATA TAGCATTCCT  
751 TGTTTAGGTG TGAATATCTT TCTCCCTCAT CTTTGAAGAT CACGGAGTAA  
801 CGAGGATTTG AAGCATCTTC AAAGTGAGGC AAGTAAATAT TATATGGATA  
851 CCTTGCTCAA GAGTGTATTA AAAGAATCTT GTTCTCTTCA TTAA

45

The PSORT algorithm predicts periplasmic space (0.571).

45

The protein was expressed in *E.coli* and purified as his-tag (Figure 96A) and GST-fusion (Figure 96B) products. The GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 96C) and for FACS analysis (Figure 96D).

These experiments show that cp6506 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 97

The following *C.pneumoniae* protein (PID 4376882) was expressed <SEQ ID 193; cp6882>:

```

5      1  MSLLNLPSSQ  DSASEDSTSQ  SQIPDPIRNR  ELVSTPEEKV  RQRLLSFLMH
51     51  KLVNPKKLI  IKKELKTLFP  LLMRKQFLIP  KRRPDILIT  PPTYTDAQGN
101    101  THNLGDPKL  LLIECKALAN  NQNALGQLLS  YNYSIGATCI  AMAGKHSQVS
151    151  ALFNPKTQTL  DFYPGLPEYS  QLLNYFISLN  L*

```

The cp6882 nucleotide sequence <SEQ ID 194> is:

```

10      1  ATGTCCTTAT  TGAACCTTCC  CTAAGCCGAC  GATTCTGCAT  CTGAGGACTC
51     51  CACATCGCAA  TCTCAAAATCT  TCGATCCCAT  TAGAAATCGG  GAGTAGTFTT
101    101  CTACTCCCGA  AGAAAAAGTC  CGCCAAAGGT  TGCTCTCCTT  CCTAATGCAT
151    151  AAGCTGAAC  ACCCTAAGAA  ACTCATCATC  ATAGAAAAAG  AACTCAAAAC
15      201  TCTTTTTCCT  CTGCTTATAG  GTAAAGGAAC  CCTAATCCCA  AAACGCCGCC
251    251  CAGATATTCT  CATCATCACT  CCCCCACAT  ACACAGACGC  ACAGGGAAAC
301    301  ACTCACAACC  TAGGCGACCC  AAAACCCCTG  CTACTTATCG  AATGTAAGGC
351    351  CTTAGCCGTA  AACCAAAATG  CACTCAAAAC  ACTCCTTAGC  TATAACTACT
401    401  CTATCGGAGC  CACCTGCATT  GCTATGGAC  GGAAACACTC  TCAAGTGTC
451    451  GCTCTCTTCA  ATCCAAAAC  ACAAACTCTT  GATTTTATTC  CTGGCCCTCC
20      501  AGAGTATTCC  CAACTCCTAA  ACTACTTAT  TTCTTTAAAC  TTATAG

```

The PSORT algorithm predicts cytoplasm (0.362).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 97A). The protein was used to immunise mice, whose sera were used in a Western blot (Figure 97B) and for FACS analysis (Figure 97C).

25 These experiments show that cp6882 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 98

The following *C.pneumoniae* protein (PID 4376979) was expressed <SEQ ID 195; cp6979>:

```

30      1  MSVNPSPGNSK  NDLWITGAHD  QHPDVKESGV  TSANLGSHRV  TASGGRQGLL
51     51  ARIKEAVTGF  FSRMSFFPRSG  APRGSQQPSA  PSADTVRSPL  PGSDARATEG
101    101  AGRNLIKKG  V  QFGMKVTIPQ  VPGGGAQRSS  GSTTLKPTRP  APPPEKTGGT
151    151  NAKRPATHGK  GPAPQPKTG  GTNAKRAATH  GKGPAPQPK  GLILQPGQSG
201    201  TSGKKRVMS  DED*

```

The cp6979 nucleotide sequence <SEQ ID 196> is:

```

35      1  ATGTCGTGTA  ATCCATCAGG  AAAATCCAAG  AACGATCTCT  GGATTACGGG
51     51  AGCTCATGAT  CAGCATCCCG  ATGTGTARAGA  ATCCGSGGGT  ACAAGTGCTA
101    101  ACCTAGGAAG  TCATAGAGTG  ACTGCCCTCAG  GAGGACGCCA  AGGGTTATTA
151    151  GCACGAATCA  AAGAAGCAGT  AACCGGGTTP  TTTAGTCGGA  TGAGCTTCTT
201    201  CAGATCGGGA  GCTCCAGAG  GTAGCCAAAC  ACCCTCTGCT  CCATCTGCAG
40      251  ATACTGTACG  TAGCCCGTTG  CGGGAGGGGG  ATGCTCGGCG  TACCGAGGGA
301    301  GCTGCTAGGA  ACTTAATTTAA  AAAAGGGTAC  CAACCGAGGA  TGAAAGTCA
351    351  TATCCACAG  GTTCTTGAG  GAGGGGCCCA  ACGTTTCATCA  GGTAGCAGCA
401    401  CACTAAAGCC  TACGCGTCCG  GCACCCGCCA  CTCCTAAAAC  GGGTGAACCT
451    451  AATGCAAAAC  GTCCGGCAAC  GCACGGGAAG  GGTCCAGCAC  CCCAGCCTCC
45      501  TAAAGCAGGT  GGGACCAATG  CTAAGCGCGC  AGCAACGCAT  GGGAAAGTCC
551    551  CAGCAGCTCA  ACCTCCTAAG  GGCATTTTGA  AACAGCTGCG  GCACTGTGGG
601    601  ACTTCAGGAA  AGAAGCGTGT  CAGCTGGTCT  GACGAGATT  AA

```

The PSORT algorithm predicts cytoplasm (0.360).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 98A). The GST-fusion protein was used to immunise mice, whose sera were used in a Western blot (Figure 98B) and for FACS analysis (Figure 98C).

These experiments show that cp6979 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 99

The following *C.pneumoniae* protein (PID 4377028) was expressed <SEQ ID 197; cp7028>:

```

1  MLLGFLCDPC CASWQCAAVA NCYDSVFMSR PEHKPNIFYI TKATRRLRLM
51  RTLAYLASLK DARQLAYDFL KDPGSLARLA KALIAPKREAL QEGNLFIFYGC
101 SNIEDILEEM RRPRLILLG FSYCQKPKAC PBGRFNDACR YDPSPHPCAS
151 CSIGTMMRLN ARRYTTVIIIP TFDILAKHLH TLKKRYPGYQ ILFAVTACEL
201 SLKMFQDVAS VMNLKGVGIR LTGRICWTFK APKLAERGVK FGVTTILEEDG
251 FEVLARILITE YSSAPFFRDF CEIH*
```

The cp7028 nucleotide sequence <SEQ ID 198> is:

```

1  ATGCTTCTAG GGTTTTGTG TGACTGCCCC TGTGCTTCGT GGCAGTGTGC
51  GGCCGTGTCT AMTGTATATG ATTCOGTATT TATGCTCTAGA CCAGAGCACACA
101 AACCTAATAT TCCTATATAT ACTAAAGCTA CAAGACGGGG TCTGCGTATG
151 AAGACGCTTG CTATCTCTGC CTCTTTAAAA GATGCTAGAC AGCTTGCCCTA
201 TGATTTTCTG AAGATCTCTG GTCTTTTATG TCGGTAGCTG AAGGCTTTGA
251 TAGCTCCTAA GGAGGCCCTTA CAGGAGGGCA ACCTATTTTT TTATGGCTGT
301 AGTAATATATG AGGATATTTT AGAGGAGATG CGTCGTCTCT ATAGATCTCT
351 TTTGTTAGGA TTTTCTTATT GTCAAAAGCC TAAGGCATGT CCTGAAGGCC
401 GTTTCAAATGA TGCTTGCTCG TATGATCCCT ACATCTCTAC ATGTGCTTCA
451 TGTCTCTATG GGAACATGAT GCGGCTGAAT GCTCGTAGAT ACACACTGTG
501 GATCATCCCT ACATTTATAG ATATCGCAAA ACATTTACAC ACTTTAAAAA
551 AGCGCTACCC TGGATATCAA ATTCTCTTTG CAGTTACTGC TTGTGAACCT
601 TCCTTAAAAA TGTTTGGAGA TTAGTCCCTCG GTAAATGAAT TAAAGGCTGT
651 GGGCATCAGA CTCACAGGAC GTATTTGCAA TACATTTAAG GCATTTAAAT
701 TAGCTGAGCG AGGAGTCAAA CAGGAGGTCA CTATCTTAGA AGAAGATGCG
751 TTTGAGGTAT TAGCAAGGAT TCTTACAGAA TACAGTAGCG CTCTCTTCCC
801 TAGAGACTTT TGTGAGATCC ATTAG
```

The PSORT algorithm predicts cytoplasm (0.1453).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 99A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 99B) and for FACS analysis (Figure 99C).

These experiments show that cp7028 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 100

The following *C.pneumoniae* protein (PID 4377355) was expressed <SEQ ID 199; cp7355>:

```

1  MKKVVTLSII FFATYCASHL SAVTVVAVPL SEAPKIQYR PVVLQFQEE
51  QSVFYSFYI PFDYGYIYPE TYGYTKNIGQ ESRECYTRFE DGTIFYECD*
```

The cp7355 nucleotide sequence <SEQ ID 200> is:

```

1  ATGAAGAAAG TCGTAACACT ATCCATTATA TTTTTCGCAA CGTATGTGCG
51  ATCAGAGCTT AGTCTGTGTA CTGATGTGGC TGTGCTCTTTA TCAGAGGCTC
101 CAGGGAAGAT TCAAGTCTGT CCGGTCGTTG GTCTGCAATT TCAGAAAGAA
151 CAGGGTCTCTG TGCCTATATG TTTTATTATAT CCTTATGACT ATGGGTATTA
201 CTATTCAGAG ACTTATGCGT ATACTAAAAA TACAGGTCAA GAAAGTCGCG
```

251 AATGTTATAC CCGATTGAA GATGGCACAA TTTTITATGA ATGCGATTAG

The PSORT algorithm predicts inner membrane (0.143).

The protein was expressed in *E.coli* and purified as a GST-fusion (Figure 100A) and a his-tag product. The proteins were used to immunise mice, whose sera were used in a Western blot (Figure 100B) and for FACS analysis (Figure 100C).

These experiments show that cp7355 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 101

The following *C.pneumoniae* protein (PID 4377380) was expressed <SEQ ID 201; cp7380>:

10 1 VHYCERTLDP KYILKIALKL RQSLSLFFQN SQSLQRAYST PYSYRIILLQ  
51 KENKEKQALA RHKICISILEF FKNLLFVHLL SLSEKNGREGC STDMAVVSTP  
101 PFNKNLWYRL LSSRFSLWKS YCPFFFLDYL EAFGLLSDFL DHQAVIKPFE  
151 LETHFYYPV SGFVAPHQYL SLQDRYFPI ASVMRLDKD NPSLTPDLIH  
201 DLLGHVPWLL HPSFSEFFIN MGRLEPKVLE KVQALPSKKQ RIQTLQSLNI  
15 251 AIVRCFWFTV ESLGIENHIEG RKAYGAVLIS SPQELGHAFI DNVRLPLEL  
301 DQIIRLPENT STPQETLFSI RHFDELVELT SKLEWMLDQG LLESIPLYNQ  
351 BKYLSGFEVL CQ\*

The cp7380 nucleotide sequence <SEQ ID 202> is:

20 1 GTGCACTACT GCGAGAGAAC CCTGGACCCA AAGTATATTA TGAAGATTGC  
51 TCTAAAGCTG AGACAATCAC TTTCCTCTGT CTTCAGAAC AGCCAATCAC  
101 TCCAACGTGC ATACTCGACC CCAATATTCCT ACTACCGAAT CATTCTACAA  
151 AAGGAAATA AAGAGAACA AGCTTAGCT CGACACAAT GCMTTCTPAT  
201 TTTAGAAATT TCACAAACT TACTCTTTGT TCATCTTCTG TCATATCAA  
25 251 AGAATCAAG GGAAGGTTGC TCCACTGATA TGGCTGTGTG AAGCACTCCC  
301 TTTTTTAATC GAAATTTATG GTATCGACTC GTTCTCTCAC GGTTTCTCTCT  
351 ATGGAAAGC TATTGTCCAA GATTTTTCTT TGAATTAATA GAAGCTTTCTG  
401 GTCTCCTTTC TGATTTCCTA GACCATCAAG CAGTCATTAA ATTCTCGAA  
451 TTAGAAACAC ATTTTTCCTA TTTATCCGCT TCAAGATTGT TAGCTCCCA  
501 TCAATACTTG TCTCTGTTCG AGGACCGTGA CTTCCTCAAT GCCTCTGTAA  
30 551 TGCGAATCTC CGATAAAGAT AATTTCTCTC TAACTCTCGA TCTCATCCAT  
601 GACCTTTTAG GCGACGTGCG TTGGCTTCTA CATCCCTCAT TTCTGAATT  
651 TTTCTATAAC ATGGGAAGAC TCTTCTACTA AGTCAATAGA AAGTACAAG  
701 CTCPTCCTAG TAAACAAACA CGCATACAAA CCCACAAAG CAATCTGATC  
35 751 GCTATTGTAC GCTGCTTTTG GTTTACTGTT GAAAGCGGAC TTATGTAAA  
801 CCATGAAGGA AGAAAGCAAT ATGGAGCGGT TCTTATCAGT TCTCTCAGG  
851 AACTTGGACA CGCTTTCATT GATACAGTAC GTGTTCTCCC TTTAGAATTG  
901 GATCAGATTA TCTGCTTTC CTTCAATACA TCAACTCCAC AAGAGACTTT  
951 ATTTTCAATA AGACATTTTG ATGAACGTGT AGAATCTCAT TCAAAATTAG  
1001 AATGGATGCT CGACCAAGGT CTGTAGAAT CAATTCCTCT TTACAATCAA  
40 1051 GAGAAATATC TTTCTGCTTT TGGGTACTTT TGCCAATGA

The PSORT algorithm predicts inner membrane (0.1362).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 101A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 101B) and for FACS analysis (Figure 101C).

45 These experiments show that cp7380 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 102

The following *C.pneumoniae* protein (PID 4376904) was expressed <SEQ ID 203; cp6904>:

-135-

1 MMNYEDAKLR GQAVAILYQI GAIKFKGKHL ASGEETFLYV DMRLVSSPS  
 51 VLQTVATLIW ELRPSFNSL LCGVFTALIT LATSISLKYV IPMVLRRKEL  
 101 QNVDFSGAIK VEGLETPGGT CLVINDMVSS GKSIETAVA LEENGAVVRE  
 151 ALVFLDRRKX ACQPLGPGGI KVSSVFTVPT LKALAIYAGK LSSGDLTLAN  
 201 KISEILBIES \*

The cp6904 nucleotide sequence <SEQ ID 204> is:

1 ATGATGNACT ACGAAGATGC AAAATTACCG GGTCAAGCTG TAGCAATTCT  
 51 ATACCAAATC GGAGCTATAA AGTTCGGAAA ACATATTCTC GCTAGCGGAG  
 101 AAGAAACTCC TCTGTATGTA GATATCGCTC TTGTGATCTC CTCTCCAGAA  
 151 GTTCTCCAGA CAGTGGCAAC TCTTATTGCG CGCCTCCGCC CCTCAITCAA  
 201 TAGTAGCTTA CTCTGGCGAG TCCCTATATC TGCTCTAACC CTAGCAACCT  
 251 CGATCTCTTT AAAATATAAC ATCCCTATGG TATTGCGAAG GAAGGAAITA  
 301 CAGAAATGTAG ACCCTCCGGA CGCTATTAAA GTAGAAGGGT TATTTAATCC  
 351 AGGACAAACT TGTTTAGTCA TCAATGATAT GGTTCCTCA GGAATACTA  
 401 TAATAGAGAC AGCAGTCGCA CTGGAAGAAA ATGGTCTCGT AGTTCGTGAA  
 451 GCATTGGTAT TCTTAGATCG TAGAAAAGAA CGGTGTCAAC CACTTGGTCC  
 501 ACAGGGAATA AAGCTCAGTT CGGTATTATC TGTACCCACT CTGATAAAG  
 551 CTTTGATCGC TTATGGGAAG CTAAGCAGTG GTGATCTAAC CCTGGCAAC  
 601 AAAATTTCGG AATTCTAGA AATTGAATCT TAA

20 The PSORT algorithm predicts cytoplasm (0.0358).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 102A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 102B) and for FACS analysis.

The cp6904 protein was also identified in the 2D-PAGE experiment.

25 These experiments show that cp6904 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 103

The following *C.pneumoniae* protein (PID 4376964) was expressed <SEQ ID 205; cp6964>:

1 MKKLIALIGI FLVPIKNTN KEHDAHATVL KAARAKYNLF FVQDVPFVHE  
 51 VIEPISPDCL VHYEGWV\*

The cp6964 nucleotide sequence <SEQ ID 206> is:

1 ATGAAAAAAT TGAATTGCTTT GATAGGGATA TTTCTTGTTT CAATAAAAGG  
 51 AATATCCCAAT AAGGAACACG ACGCTCACGC GACTGTTTTA AAAGCGGCCA  
 101 GAGCAAAAGTA TAAATTGTTC TTGTGTCAGG ATGTTTTCCT TGTACACGAA  
 151 GTTATCGAGC CTATTGTCTC CGATTGCTGT GTACATTATG AAGGGTGGGT  
 201 TTGA

The PSORT algorithm predicts inner membrane (0.091).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 103A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a

40 Western blot (Figure 103B) and for FACS analysis (Figure 103C).

These experiments show that cp6964 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 104

The following *C.pneumoniae* protein (PID 4377387) was expressed <SEQ ID 207; cp7387>:

-136-

```

1  LNFPAKIDHNH LYL/CLADLG VACPILSTDC LPNYSKASH EVLVYSKFRG
51  ISGEPRLAT SGNDTYSIV SLPIGLRYEV TSPSGRHDNP IDMEVAPKIG
101  AVLSHSTREA KEIPGSSKDY AFFSLTARES LMISEKLAMT FQVSEVIQNC
151  YSQCTKVKTK NLKEQYRHS HNTGFLSVK SAF*

```

5 The cp3787 nucleotide sequence <SEQ ID 208> is:

```

1  TTGAATTTTG CAAAGATTGA TCACAATCAT CTCTACCTTA CATGTTGGGG
51  AGATCTTGGT TAGCTTGTCT CTATACCTTC TACAGATTGT CTACCTAATT
101  ATAGCAGGAA AGCATCTCAT GAGGTCTCTG TTTATAGTAA ATTTAGATGC
151  ATTTCTGTGAG AGCCATCTCG ACTTGCAACT TCAGGAAATG ACACATATTA
201  TTCTATAGTA AGTTTACCTA TAGGACTTCG TTACGAAATG ACTTCACCAT
251  CAGGACGTCA TGATTCAAT ATTGATATCG ATGTAGCTCC AAAGATAGGT
301  GCAGTACTCT CTCATGGGAC ACGAGAGGCT AAAGAGATCC CAGGATCTTC
351  AAAAGACTAT GCATTTTATA GCTTGACCTG TAGAGAAATG TTAATGATTT
401  CTGAAAAGCT TCGGATGACT TTCCAAATTA GOGAAGTTAT TCAGAAATGT
451  TATTCACAAT GTACTAAAT AACGAAAAC ATTTTAAAG AACAGTATAG
15  551  GCACCTTACC CACAATACAG GGTTTGAGTT AAGCGTCAG TCACATCTCT
551  AA

```

The PSORT algorithm predicts inner membrane (0.043).

10 The protein was expressed in *E.coli* and purified as a his-tagged-fusion product (Figure 104A) and also as a GST-fusion (Figure 104B). The recombinant proteins were used to immunise mice, whose sera were used in a Western blot and for FACS analysis (Figure 104C; his-tagged).

These experiments show that cp3787 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 105

25 The following *C.pneumoniae* protein (PID 4376281) was expressed <SEQ ID 209; cp6281>:

```

1  MFLQFHPPIV FSDQSLSLPL YLQKSSGIIIE KCSNIVEHYL HLGQDTSVII
51  TCVSGATFLS VDMALPISK EKLIKILSVI LILPLIALAF IKIVLRILIF
101  FKYAGLILDV KKEDLKKTLF PDQENLSLPL PSPPTLKKIH ALHILVRSGK
151  TYNELIQEGF SPTKITDLQQ AOSPKQDIGF SVNSLLPNFY PHSLVSVPMI
30  201  SGGERALNYH KEQGEEMAVK LKTMQACSVF FRSLLHPSMQ TKDKKAGPGL
251  LTTTTFKIYF L*

```

The cp6281 nucleotide sequence <SEQ ID 210> is:

```

1  ATGTTTCTTC AGTFTTTTCA TCCTATAGTC TTCTGGGATC AGTCCITATC
51  TTTTCTTCTCT TACCTAGGAA AAAGCTCTGG CATATTAGAA AAATGTPCCA
35  101  ATATCGTTGA ACACATATTA CATTTGGGAG GAGACACTTC TGTTATCATC
151  ACAGGAGTTT CTGGAGCTAC CTCTTATATCT GTTGATCATG CCGTCCCAAT
201  CTCGAAATCT GAAAATAATA TAAAATTTCT CTCTATATT TTAATCTCTC
251  CTCTGATCTCT AGCTCTCTTT ATTAAGATCG TTTTACGCAT TATCTTATTC
301  TTCAAGATATC GTGGTCTAAT CCTAGATGTT AAGAAGGAGG ATTTGAAAAA
40  351  AACACTTACA CCGTACCAAG AAAACCTCAG TCTCTCTTTA CCATCTTCTA
401  CAACATTAAA GAAATTCAT GCGCTACACA TTTTAGTCGG TTCTGGAAAA
451  ACCATATAAG AGCTTATACA AGAAGGGTTT TCTTCTACTA AAATCAGACA
501  TCTTGGTCAA GCTCTCTCAC CAAAGCAAGA TATTGGCTTC TCTTATAATT
551  CCCTTCTCCC TAACTTCTAT TTTTATCTCT TGGTATCTGT TCCAAATATT
45  601  TCAGGCGAGG AACGGGCTCT TAATTATCAT AAAGAACAAC AAGAGGAAT
651  GGCCTGTAAA TTAATAACAA TGCAAGCGTG TTCTTTTGTG TTCCGATCCC
701  TGCATTATAC TCAATGCAA ACGAAGGACA AAAAGGCTGG ATTTGGAGTA
751  CTGACGTTT TCCCTTGGAA AATCTACCCC CTATAA

```

The PSORT algorithm predicts inner membrane (0.5373).

50 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 105A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 105B) and for FACS analysis.

These experiments show that cp6281 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 106 and Example 107

- 5 The following *C.pneumoniae* protein (PID 4376306) was expressed <SEQ ID 211; cp6306>:

```

1  MGNHETYIHP  GVLPSSSHAQD  VSRSTVYPSR  SFIMRRLMG  WNFNRVPSKS
51  SEQLMDGHRI  PLIFFPGKHP  TISILNVNRF  SWSLIFYNG  RGF*
```

The cp6306 nucleotide sequence <SEQ ID 212> is:

```

1  ATGGGAACC  ATGAGACCTA  TATACATCCA  GGAGTGCTCC  CGAGTAGTCA
10  TGCTCAGGAT  GTTAGCAGAT  CTACAGTTTA  CCCAGTCGA  AGTTTATCA
101  TGAGACGTAT  GCTCATGGGC  TGAATTTCA  ATCGTGTCC  CTCGAAGAGC
151  TCCGAGCAGT  TAATGGATGG  TCAATCCGCA  CCTCTATAT  TTTTGGGAA
201  GCATCATCCT  ACTATATCTA  TTTTAAATGT  CAATAGATT  TCTTGGCTCT
251  CCATTTTTTA  CAATGGAGAA  AGGGGGTTTT  GA
```

- 15 The PSORT algorithm predicts cytoplasm (0.167).

The following *C.pneumoniae* protein (PID 4376434) was also expressed <SEQ ID 213; cp6434>:

```

1  MSESINKSIH  LEASTPFFIK  LTNLCESRLV  KITSLVISLL  ALVGAGVTLV
51  VLFVAGILPL  LFLVLLEILL  ITVLVLLFCL  VLEPYLIEKP  SKIKELPKVD
101  ELSVVETDST  L*
```

- 20 The cp6434 nucleotide sequence <SEQ ID 214> is:

```

1  ATGTCTGAAA  GTATTAAACG  AAGCATTCAT  TTAGAAGCCT  CTACACCAAT
51  TTTTATATAA  TTAACGAATC  TCTGTGAAG  TAGATTAGTT  AAGATCACTT
101  CTCTTGTGAT  TCTCTATATA  GCTTTAGTGG  GTGCGGGAGT  CACTCTTGTG
151  GTTTTATTTG  TAGCTGGGAT  CCTTCTTTA  CTTCCTGTAT  TCATCTTAGA
201  AATTATTTTA  ATAAACCGTC  TTGTCTTGCT  TTTTGTGTTG  GTATTGGAAC
251  CTTATTTAAT  AGAAAAACCT  AGTAAATATA  AGGAACCTACC  TAAAGTAGAC
301  GAGCTATCTG  TAGTAGAAAC  GGACAGTACT  CTTTAA
```

The PSORT algorithm predicts inner membrane (0.6859).

- 30 The proteins were expressed in *E.coli* and purified as his-tag products (Figure 106A; 6306 = lanes 2-4; 6434 = lanes 8-10). The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 106B & 107) and for FACS analysis.

These experiments show that cp6306 & cp6434 are surface-exposed and immunoaccessible proteins, and that they are useful immunogens. These properties are not evident from the sequences alone.

### Example 108

- 35 The following *C.pneumoniae* protein (PID 4377400) was expressed <SEQ ID 215; cp7400>:

```

1  MRVRRFFCLF  FLGFLGSFHC  VAEDKGVDLF  GVWDNDQITE  CDDSYMTEGR
51  EEVEKVVDVA
```

The cp7400 nucleotide sequence <SEQ ID 216> is:

```

1  GTGAGAGTTA  TGAGATTTT  TTGCTATTT  TTCTTTGGT  TCTTAGGATC
40  TTTTCATTGT  GTTGTGAAG  ACAAGGGCGT  GGAATTTATT  GGAATCTGGG
101  ACGATTAACCA  AATTACAGAG  TGTGACGATA  GTTACATGAC  AGAGGGTCTG
151  GAAGAGGTTG  AAAAGGTAGT  GGACGCTTAG
```

The PSORT algorithm predicts periplasmic space (0.924).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 108A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 108B) and for FACS analysis.

These experiments show that cp7400 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 109

The following *C.pneumoniae* protein (PID 4376395) was expressed <SEQ ID 217; cp6395>:

```

1  MENAMSSSFV YNGPSWLKLT SVAQEVFKKH GKGIQVLLST SVMLFIGLGV
51  CAPTFPQYLI VFVLITALLM LAISLVLFLL IRSVRSSMVD RLWCSEKGYA
101 LHQHENGPFLL DVKRVQQLL RSPYIKVRAL WPSGDIPEDL SQAVALLLSP
151 WTFPSSVDVE ALLPSPQEKKE GKIIDPVLPK LSRIERYSL VFLSAFTLDD
201 LNEQGVNPLM NNEBFLFFIN KKAREHGIQD LKHEIMSSLE KTGVPFLDPM
251 SPQVSQAMPS VYRYLRQRDL TTSELRCPHL LSCFGQDVVH CLASFENPKD
301 LADSDFLBAC KNVWGEFIS ACKKALLKNP QGISIKDLKG FLVR*

```

The cp6395 nucleotide sequence <SEQ ID 218> is:

```

1  ATGGAGAATG CTATGTCATC ATCGTTTGTG TATAATGGCG CTTCTGGGAT
51  TTTAAAAACG TCAGTAGCTC AGGAGGTATT TAAAAAGCAC GGTAAAGGGA
101 TTCAGGTCTCT CTTAAGTACT TCAGTGATGC TTTTATAGG TCTTGAAGTC
151 TGTGCGCTTCA TATTTCCTCA ATATCTGATT GTTTTCTGTT TGACTATAGC
201 TTTGCTTATG CTCGCTATAA GCTTGGTATT GTTCTCTCTA ATACGTTCTG
251 TACGCTCTTC AATGGTAGAT CGTTTGTGGT GTTCTGAAAA AGGATATGCT
301 CTTCTCAACG ATGAGAACGG GCCTTTTGTG GATGTGAAGC GTGACAGACA
351 AATTCTTCTA AGATCAACCT ATATTAAAGT TCGGGCTTTA TGGCCGCTCG
401 GAGATATCCC TGAGGATCCT TCACAAGCTG CGGTTCTATT ACTTTCTCCT
451 TGGACTTTCT TTTCACTCCG TGAATGAGAG GCTTTATATC CAGATCCTCA
501 AGAAAAAGGAG GGTAAAGTATA TAGATCCTGT GCGCCCTAAG TTTGTAGAGA
551 TAGAGAGAGT CTCACCTTTA GTGCTTTTGA GTGCATTTAC TTTGGATGAC
601 TTAACCGAAC AGGAGACTCA TCCTTTTGAT AATAATGAGG AATTTTATTT
651 TTTTATATAAT AAGAAAGCGC GTGAGCATGG GATTCAGAGT TTAACAACAG
701 AGATTATGTC TTCGTTAGAG AAAACAGGAG TGCCATTAGA CCCCCTAATG
751 AGTTTTCACG TTTCAACAAG CATGTTTTCCT GTATATCGCT ACTTGAGACA
801 AAGGGATTTA ACGACTTCAG AATTAAAGAT TTTTCAOCTC TTAAGTTGTT
851 TTAAGAGGGA TGTGGTTCAT TGTTTAGCTT CATTTGAAAA CCTTAAGAT
901 TTAGCAGATT CTGACTTTTT AGAAGCTTGT AAGAACGTGG AATGGGGTGA
951 GTTTATTTCG GCATGTGAGA AGGCTCTTTT AAGAAATCCG CAAGGAATTT
1001 CCATTAAAGA TCTAAACAAA TTTTATGTGA GGTAA

```

The PSORT algorithm predicts inner membrane (0.6307).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 109A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 109B) and for FACS analysis.

These experiments show that cp6395 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 110

The following *C.pneumoniae* protein (PID 4376396) was expressed <SEQ ID 219; cp6396>:

```

1  MIEFAFVPHY SVTADRIHDR MACRMNKLST LAITSLCVLI SSVCIHTGL
51  CIGSTVGTYA FVVGIIISVL ALVACVFPLY FFFVSSSEKHE CASSQEFRL
101 PIPAVVSALR SYEYISQDAI HDVIKITMQL STLSSLDDPE AFFLEPPYFN
151 SLIVHMSMKE ADRLSREAPL ILLGEITWKD CETKILPWLK DPNITPDDFW
201 KULKDHFDLK DFKKRIATWI RKAYPEIRLP KKHCLDRSY KGCCKFLLS

```



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251 ENDVQYQRLH HKVCYFSGEF PAMVLGLGSE VPMVLGLPKV PKDLTWEMFM  
301 ENMPVLLQSK REGHKKISLE DVASL\*

The cp6396 nucleotide sequence <SEQ ID 220> is:

5 1 ATGATCGAGT TTGCTTTTGT TCCTCATACC TCCGTGACAG CGGATCGGAT  
51 TGAGGATCGC ATGGCCCTGTC GCATGAACAA GTTGTCTACT TTAGCAATTA  
101 CAAGTCCTTG TGTATTGATC AGTTCAGTTT GTATTATGAT TGGGATTTTA  
151 TGCATTCTCTG GAACGGTGG GACCTATGCA TTTGTGTAG GAATTAATTT  
201 TCTCTGTGCTT GCTTTGGTAG CATGTCTTTT CTTCTTTTAT TCTCTTTAT  
10 251 TTTCTTCTGTA GGAATTTAAG TGTGCTCTTT CGCAGGAGTT TCGTTTPTTG  
301 CCTATACCAG CTGTGGTTTC TGCATTGCT GTCTATGAAT ACATTTCTCA  
351 GGACGCTATC AATGACGTTA TAAAGATAC GATGCAGTTG TCTACCCCTT  
401 CTCTCTCTTT AGATCCCGAA GCTTTTCTCT TAGAATTTC TATTPTTAAC  
451 TCTTTGATAG TGAATCATTC GATGAAGGAA CGCGATCGTT TGCTCTGAGA  
15 501 GGCCTTTTGT ATTTTATTAG GTGAGATTAC TTGGAAGGAT TGTGAACAA  
551 AAATTTTGCC ATGGTTGAAA GATCTTAATA TCACTCCTCA TGATTTCTGG  
601 AAGCTATTAA AAGACCATTG CATTTAAAG GACTTTAAGA AGAGGATCGC  
651 CACTTTGGATA CGGAAGCCCT ATCCAGAAAT TAGATTACCG AAGAGCATT  
701 GTTTAGATAA GTCTATCTAT AAGGGGTGTT GTAACTTTT ATTACTPTCT  
20 751 GAGAATGATG TGCAATATCA GAGGTATTA CATPAGGTCT GTATTTCCTC  
801 TGGGGAGTTT CCTGCCATGG TTTTAGGTTT GGGAGGTGAA GTGCTATGG  
851 TGTTAGGACT CCTTAAGGT CCCAAGGATC TTACCTGGGA GATGTTTATG  
901 GAAATATGTC CTGTCTCTCT GCAAAGCAA AGAGAGGGGC ATTGAAAAAT  
951 CTCTTGGAA GACGTAGCCT CTCTTTAA

The PSORT algorithm predicts inner membrane (0.6095).

- 25 The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 110A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 110B) and for FACS analysis.

These experiments show that cp6396 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### 30 Example 111

The following *C. pneumoniae* protein (PID 4376408) was expressed <SEQ ID 221; cp6408>:

51 MNTSLKRLPK SHFDVVSFL RPHLKKTRR SLKESSISLD QIAQIEDIAI  
51 QDLIKKQKAA GLSFITDGEF RRAVWHYDEM WCFHGVGHR ATGVVFDDGE  
101 RAMIDTDLVL DKISVSHHPE VDHFFVKAL EDEFTAKGT LPAPAQFLKQ  
35 151 MIFPNNIEVT KKFYPTNQEL IEDIVAGYRK VIRLDVAGC RTQLDLDCTR  
201 GGLVDPRVCS WYGDIEKQL DLIQYLLTN NLVIAERPPD LVVNLHVCRG  
251 NYHSKFPAFG SYDFIAKPLF EQTNVDGYVL EFDHERSGDF SPLTFISGEG  
301 TVCLGLVTSK TPTLENKDEV IARIHQADY LPLRLSLSP QCGFASCBIG  
351 NKLTEEEQWA KVALVKEISE EVWK\*

- 40 The cp6408 nucleotide sequence <SEQ ID 222> is:

1 ATGAATACTT CACTAAAAAG ACCCTGAAA TCTCATTTTG ATGTTGTCCG  
51 TAGTTTTTTG CGTCCCTGAGC ATTTAAAAAA AACTAGAGAA AGCCTTAAG  
101 AAGGCTCTAT TTCTCTAGAT CAACCTATGC AAATTTAGGA TATCGCTATC  
45 151 CAGATTTTGA TCAAAAAACA AAAAGCAGCA GGTCTTTCTT TTATTACTGA  
201 TGGAGAAATC CGCAGAGCTA CGTGCAATTA CGACTTTCTG TGGGGTTTTC  
251 ATGGCGTAGG TCACCAACAGA GTACACGAAG GAGPTTTCTT TGAATGAGAA  
301 CGCGCTATGA TCGATGATAC CTAATCTGACA GACAAGATCT CTGATCTCA  
351 CCACCAATTT GTGGATCACT TTAATTTTGT AAAAGCTCTA GAGATGAAT  
50 401 TTACGACTGC AAAGCAAACT CTTCTGACAC CGGCACAGTT TTTAAAGCAG  
451 ATGATCTTCC CTAATATAT ATAGGCTACA CGTAAATTTCT ATCTACAAA  
501 TCAGGAGCTA ATTGAAGATA TTGTTGCAGG TTATCGTAAA GTCATTTCGG  
551 ATCTTTATGA TGCTGGCTGC CGCTATCTCC AATTAGATGA CTGATCTCGG  
601 GGAAGTTTAT TAGACCCCTG AGTCTGTCTG TGATATGGTA TCGATGAAA  
651 AGGTCTTCAA GATCTGATTC AACAATATCT TCTGATTAAT AATCTTGTA  
55 701 TTGCAGATCG TCCCGATGAT CTAGTCGTGA ATTACATGAT ATGCGGTGG

751 AACTACCACCT CAAAATTCCTT TGCTAGTGGT AGTTATGACT TTATTGCAAA  
 801 GCCCCTATTC GAACAAACAA APTAGAGCGG CTACTATTTTA GAOTTTGATC  
 851 ATGAGCGTTC TGGAGACTTC TCCTGCTCCA CCTTCATTTTC TGGAGAAAAA  
 901 ACTGTCTGCT TAGGCTTGCT TACCAGACAA ACCCTACAC TTGAAATATA  
 951 GGATGAGCTC ATTGCTCGCA TCATCAACG AGCAGACTAC CTGCCCTTGG  
 1001 AAGAGCTCTC TCTATGTTCA CAGGTGCGTT TCGCTCATG TGAAATAGGA  
 1051 AATAAATTA CAGAGAGAGA GCATGGGCT AAAGTTGCTC TAGTAAAGAA  
 1101 AATTTCGGAA GAGTTTGA AATAA

The PSORT algorithm predicts cytoplasm (0.2171).

- 10 The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 111A) and also as a his-tagged product. The his-tag protein was used to immunise mice, whose sera were used in a Western blot (Figure 111B) and for FACS analysis.

These experiments show that cp6408 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

# Example 112

The following *C. pneumoniae* protein (PID 4376430) was expressed <SEQ ID 223; cp6430>:

1 MKLYSISSDV DTPWIFQLMS KVDVSLFLGG NRKVVSVIM QEPNLIIGKV  
 51 ENVRISTIVK ILKILSLFLP PLILIALALH YFLHAKYANH LLVSKILERA  
 101 PQYVPIPGRS GDTASHYKLT TLVPVSQKNL QAMGSNPLEV EALRTTKPS  
 151 PFCVPKVRQ IILSHGIRF SLDLQLADD INLDSVSWPT EYLNSTMDPC  
 201 SKADKRVION VQNLRTGYI NSVGKRSLLK FMLQHLFIDG ITCENPEALP  
 251 NNTSGRLTLF PSURYIYSHF TPQNPTIWPQ VFFRQQLDE DRGGGFELLE  
 301 QLQELGVRFP ICPSSQPDNP NFQGFQGI RI YWEDSYQPNK EW\*

The cp6430 nucleotide sequence <SEQ ID 224> is:

25 1 ATGAACTTT ATAGCATCTC TTCAGATGTA GATACACCTT GGATATTTC  
 51 GCTTAGTCA AAGTAGATT CTATCTTTT CTTAGGCGGG AATAGATCA  
 101 AGGTGTATC TATATGATG CAGAAACCTA ACTTATTTAT TGGAAAGTA  
 151 GAAACGTTT GGAATCTCAC AATAGTGAA ATATTAAGA TTTTATCCTT  
 201 CTTAACTTC CCTCTGATTT TAATCGCTTT AGCCCTACAC TATTTCTPAC  
 251 ATGCTAATA TGCCTATCAC TTAATCTGAT CTAGATTTT AGAAAGACT  
 301 CCTCAGTATG TGCTATTTCC TGCTCGTTCA GGAGACACGG CTTCTCATTA  
 351 TAAATTAACA ACATTGGTTC CAGTATCCCA AAAAATCTA CAGCTATGG  
 401 GATCAAAATC TCTAGAATT GAAGCGGCTC TTGACACTAC AAAACCTCT  
 451 TTTTCTGTG TACCTGCAAA ATACCGTACG ATTATATTTT CAGCTACGG  
 501 CATTCGCTTT TCTTTAGATC TTGAACAACT TGCTAGTGAC ATTAATTTAG  
 551 ATTCTGTTTC CTGGCTTACG GAGTATCTTA ACTCTACTAT GGATTTTTCG  
 601 AGCAAGCGAG ATAAAGTGT TATACAGAAT GTACAAATC TGGCGACAGG  
 651 AACTTACATA AATCTGTAG GAAAGCGTAG CCCTTTTAAA TTCAATTTAC  
 701 AGCACTTATT TATGATGGG ATCACACAAG AAAACCTCGA AGCCCTTTCT  
 751 AACAAATCAT CTGGAAGACT GACTCTATT TCCTAGTGTG GTTATATCTA  
 801 TCTCTATTTC ATCTCACAAA ATCTTCAAT ATGGCCGCAA GTCTTTTCTA  
 851 GACAAGGTCC TCTAGATGAA GATCCGAGGAG GAGGATTTGA GATCTTAGAG  
 901 CAATTACAG ATTAGGAGT TAGGTTTCCA ATTTGCCCTC CTCAAGGACC  
 951 AGACAATCCT AATTTCACAG GTTCTCAAG GATTCTGATC TATTTGGGAG  
 1001 ATTCCTATCA ACCCAATAG GAGTTTAA

The PSORT algorithm predicts inner membrane (0.5140).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 112A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 112B) and for FACS analysis.

- 50 These experiments show that cp6430 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

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**Example 113**

The following *C.pneumoniae* protein (PID 4376439) was expressed <SEQ ID 225; cp6439>:

```

1 MSYDTLFPKNI EKEDSVHKIC NEIFALVPRL NTIACTEALI KNLFPADIHV
5 51 HLPGETITPQL AWILGVKNKF LKWSYNSWNT HRLLSPKNNF KQYSNIFRNF
101 QDICHEKDPD LSVLQYNILN YDFNSFDRVM ATVQGHFRFP GGIQNEBDLL
151 LIFNNVLQQC LDDTIVYVEV QQNIHLAHVL YPSLPEKHAR MKFYQILYRA
201 SOTFSKHGIT LRFLNCFNKT FAPQINTQEP AQEAVQWLQE VDSFTFPLFV
251 GIQSAGSESA PGACPKRLAS GYRNAYDSGF GCEAHAGEEI ETRTIFSSAK
301 VNPEGLIEIT RVTFSLSLRK QPSSLPRVTV CQLG*
```

10 The cp6439 nucleotide sequence <SEQ ID 226> is:

```

1 ATGCTCTTATG ATACGTTATT CAAGAATCTT GAAAAGGAAG ATTCTGTACA
5 51 TAAGATATGC AATGAGATCT TTGCATTAGT ACCACGACTC AATCAATCG
101 CTTGCACCGA AGCTATCATC AAAAACCTCC CCAAGACGAC TAGCCATGTA
15 151 CACCTTCTCG GGACCATAAC ACCTCAATTA GCTTGAGTTT TAGGTGTGAA
201 AATATGGGTC TTAAATGGT CTGATAATCT TCGGACCAAT CATCGATTAC
251 TTCTCTCCTAA GATTCCTCAT AAACAACTCT CCAATTTTTT CGGAACCTTT
301 CAAGATATCT GTACGAGAAA GATCCGGAT TTAAGTGTAT TACAATATAA
351 TATCTTAAAT TACGATTTTA ATAGCTTTGA TAGAGTGATG GCTACAGTAC
401 AAGGACATCG CTTTCTCTCT GGAGGAATCC AAAATGAGAA AGACCTTCTT
20 451 CTCATTTTCA ATAACTATCT CCAGCAATGT CTGAGCGMTA CTATCGTGT
501 TACTGAAGTA CAACAAAATA TCCGCCCTTG CCAATGTTTG TATCCTTCAT
551 TACCTGAAAA GCACGCGCGT ATGAAGTTTT ATCAAACTTT GTATCGTGTCT
601 TCGCAAAAGT TTTCAAAACA CGGAGTTACT TTACGATTTT TAAACTGCTT
25 651 CAATAAACA TTGCTCCAC AATTAACAC ACAAGAACCT GCCCAAGAG
701 CTGTTCAATG GCTCCAGAGT GTTGTATCTA CATTTCTCTG TCTATTGTGA
751 GGGATACAA CCGCAGGATC AGAATCTGCG CCCGAGCCT GCTCCAAGCG
801 ATTAGCTTCT GGATATAGAA ATGCTTATGA CTCAGGGTTT GGTGTGTGAAG
851 CTCATGCTGG AAGAGGCATA GAGACCCGGA CTATTTTTTC GTACAGCTAG
901 GTAATCTCAG AGGGAATGAT CGAGATAAC CAGTGACTIT TCTCGTCTCT
30 951 TAAACGAAA CAGCCATCTA GTTTTACCCT AAGAGTTACT TGCCAGTTAG
1001 GATAA
```

The PSORT algorithm predicts cytoplasm (0.1628).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 113A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 113B) and for FACS analysis.

These experiments show that cp6439 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

**Example 114**

The following *C.pneumoniae* protein (PID 4376440) was expressed <SEQ ID 227; cp6440>:

```

40 1 LQSARRHLNT IFILDFGSQY TVVLAKQVRK LFVYCEVLFW NISVQCLKER
51 APLGIILSGG PHSVYENKAF HLDPEIYKLG IPILAICYGM QLMARDFGGT
101 VSPVGGEFGY TPIHLYPCBL FKHIIVDCBSL DTEIRMSHRD HVTPIPEGFN
151 VIASSTQCSI SGIENTKQRL YGLQFHEPVS DSTPTGNKIL ETFVQICSA
201 PTLWNPLYIQ QDLVSKIQDT VIEVFDEVAQ SLVQVNLQAG TIYSDVIESS
45 251 RSGHASEVIK SHHNVGLLPK NLEKLILVEPL RYLFKDEKVI LGEALGLSSY
301 LLDRHPPFPGL GLTIRVIGET LPEYLAILLR ADLIFIEELR KAKLYDKISQ
351 AFALFLPIKS VSVKGDCEYS GVTIALRAVE STDFTMTGRWA YLPCDVLSSC
401 SSRIINEIPE VSRVVDYDSD KPPATIEWE*
```

The cp6440 nucleotide sequence <SEQ ID 228> is:

```

50 1 TTGCAGAGTG CAGGAGGACA TTGTGAACAC ATATTTTATTC TAGATTTTGG
51 ATCTCAATAT ACTTATGTAT TAGCAAGACA AGTGGCGAAG TTATTTGTAT
101 ATTGCGAAGT TCTTCCCTGG AATATCTCTG TGCATATGTT AAAAGAAAG
151 GCGCCCTTGG GGATCATTTCT CTCAGGAGGT CCTCACCTCG TCTATGAAA
```

201 CAAGGCTCCA CMTTAGATC CTGAATCTA TAAACTTGGC ATTCCAAATTC  
 251 TAGCTATTCTG CTATGGCATG CAGCTTATGG CPAGAGATT TGGAGGGACT  
 301 GGAAGCCCTG GTGTAGGAGA ATTTGGATAT ACGCCCACTC ATCTGTATCC  
 351 TTGTGAGCTC TTCAAAACACA TCGTCGACTG CGAATCTCTA GACACAGAGA  
 401 TTGGGATGAG CCATCGGGAAT CATGTTACGA CAATTCCTGA AGGATTTAAT  
 451 GGAATCGCAT CCACCTCACA ATGCTCGATC TCAGGAATAG AAAATACCAA  
 501 ACAACGGTTG TACGGGCTGC AACTTCATCT CGAGGTTTCT GACTCCACTC  
 551 CAACGGGAAA TAGATCTCTA GAACCTTTTG TTCAAGAGAT CTGTTCTGTCT  
 601 CCCACACTAT GGAATCCCTT GTATATTCAG CAAGAACCCTG TAAGTAAAT  
 651 TCAAGATACC GTTATTTGAG TATTTGATGA AGTCGCTCAG TCATTAGACG  
 701 TACAATGGTT AGCTCAAGGA ACCATCTACT CAGATGTTAT TGAGTCTCTA  
 751 GCGCTTGGAC ATGCTCCGGA AGTAATAAAA TCACATCATA ATGTAGGGGG  
 801 GCTTCCAAAA AATCTTAAGC TGAAGTTAGT CGAGCCCTTA GGTATTTTAT  
 851 TTAAGATGA AGTTGGAAT TTAGGAGAGC CCCTAGGACT TTCTAGCTAT  
 901 CTCCTGGACA GGCATCCCTT TCCTTGGACT GGCTTGACAA TTCGTGTGAT  
 951 TGGAGAGATC CTTCCTGAAT ATCTAGCCAT TTTCAGACGG GCGGACCTCA  
 1001 TCTTTATAGA AGAGCTTAGG AAGCAAAAC TCTACGATAA AATCAAGCAA  
 1051 GCGTTTGCTC TATTCTCTTC TATAAATCA GTATCTGTAA AAGGAGATCT  
 1101 TAGAAGCTAT GGTATACCA TAGCATTCAG TGCTGTATGA TCTACAGAT  
 1151 TCAATGACAG GAGATGGGCT TACCTTCCAT GCGATGTTCT CAGTCTGTCT  
 1201 TCAACCGGAA TTAATTAATGA AATACCCGAG GTAAGCCGAG TGGTCTATGA  
 1251 TATTCTGAC AAGCCACGAC CAACTATAGA ATGGGAATAG

The PSORT algorithm predicts cytoplasm (0.0481).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 114A) and also as a his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 114B) and for FACS analysis.

These experiments show that cp6440 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 115

The following *C.pneumoniae* protein (PID 4376475) was expressed <SEQ ID 229; cp6475>:

1 MNTVTFSPFL QKSFSLFLL KLDSPFFFG TRQQLVITP TNIRLAAKRR  
 51 GCKVSTIEKI IKILSFLLP LVIIAIFILRY FLHKKFDKQF LCIPKVISNE  
 101 DEALLGSRPQ AVEKAVREIS PAFFSIPRKY QLIRIDTPKD DAPSLFPIG  
 151 IELIKDLICI DFLKQSNLPI KREMDPLGHP EKKALPDSIC SIEKDQWMS  
 201 LESKLLITH FLKYLEVSGI BQLNPGFNP NRGYFVSEIS TAKIHPHQHG  
 251 RYGPTRSSGP IMKEI\*

The cp6475 nucleotide sequence <SEQ ID 230> is:

1 ATGAATACCT ATACCTTCTC TCCTCACTTT CAGAAAGCT TCAGCCTATT  
 51 TCTTTTAGAA AATATAGACT CTACTTTTCT CTGTGAGGG ACTGTACAC  
 101 AAATCTTAGT CATCAACCA ACCAATATTA GATTCAGGC TAAAAAAGA  
 151 GGGGTAAAGG TTCTACTACT AGAAAAGATA ATCAAGATCC TCTCTTTTAT  
 201 CTGCTGCCCC CTAGTTATCA TTGCTTTTAT ACTTCGCTAT TTCTTACAGA  
 251 AGAAATTCGA TAAACAGTTC TTGTGTATCC CAARAAGTCAT TTCTAACGAA  
 301 GACGAAGCTC CTCTTGGATC TAGACCAACA CAGGTTGAAA AAGCAGTTCG  
 351 AGAAATATCT CCAGCTCTCT TCTCTATACC AAGAAAATAC CAACTTATTA  
 401 GAATCCACAC TCTCAAGA GACGCTCCCT CAATCTCTTT CCCTATAGGC  
 451 ATAGAGATCA TTCTCAAGA TTTATGTATG GATACACTCA AGCAATCTAA  
 501 TCTTTTCTTT AAAAGAGAAA TGGATTTCTT AGGTCACTCA GAAGAAAGA  
 551 CATATATCGA CTCGATATGT TCTATAGAAA AAGATCAAGA ATGGATGAGC  
 601 TTGGAAAGTA AAAAATCTTT AATCAGCAC TTCTCTAAGT ATCTCTTTGT  
 651 CTCGGGAATC GAACCACTAA ATCCAGGCTT TAACCCAGAG AATGGCGGTG  
 701 GGATTTTCTC AGAAATAAGT ACAGCAAGA TCCATTTTCA TCAGCAGGT  
 751 CGATATGGGC CAATCCGTTT TTCGGGACCC ATCATGAAGG AAGATATA

The PSORT algorithm predicts inner membrane (0.5373).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 115A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 115B) and for FACS analysis.

These experiments show that cp6475 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 116

The following *C.pneumoniae* protein (PID 4376482) was expressed <SEQ ID 231; cp6482>:

```

1 MLVELEALKR EFAHLKDQKP TSDQEITSLY QCLDHLEFVL LGLGQDKFLK
51 ATEDEDVLFE SQKATDAMNA LITKARDVLG LGDIGAIYQT IEFLGAYLSK
101 VNRRAPCIAS EIHFLLKTAIR DLNAYLLDF RWPLCKIEEF VDWGNDVCBI
151 AKRKLCTFEK ETKELNESLL REEHAMEKCS IQDLQRKLSD IIEHLHDVSL
201 FCFSKTFSQE EYQKDCILYQS RLRYLLLLLYE YTLCLKTSTD FQEQARAKEE
251 FIREKPSLLE LKGGIKQTKR LEFATAKSKL ERGCLVMRKY EAAKSHLDS
301 MFEETVKSP RKDTE*

```

The cp6482 nucleotide sequence <SEQ ID 232> is:

```

1 ATGCTAGTAG AGTTAGAGGC TCTTAAAGA GAGTTGCGC ATTTAAAGA
51 CCAGAAGCCG ACAAGTGACC AAGAGATCAC TTCACCTTAT CAATGTTTGG
101 ATCATCTTGA ATTGCTTTTA CTCGGGCTGG GCCAGGACAA ATTTTAAAG
151 GCTACGGGAA GTGAAGATGT GCTTTTGTAG TCTCAAAAG CAATCGATGC
201 GTGGAATGCT TTATTGACAA AAGCCAGAGA TGTTTAGST CTGGGGACA
251 TAGGTGCTAT CTATCAGACT ATAGRAATCT TGGGTGCCAT TTTATCAAAA
301 GTGAATCGGA GGGCTTTTGT TATTGCTCG GAGATACATT TTCTAAAAAC
351 AGCAATCCGA GATTTGAATG CATATTACCT GTTAGATTTT AGAUGGCCCT
401 TTTGCAAGAT AGAAGAGTTT GTGGATTGGG GGAATGAATT TGTGAAATA
451 GCAAGAGGGA AGCTATGCAC TTTTGAAAAA GAAACCAAGG AGCTCAATGA
501 GAGCCTTCTT AGAGAGGAGC ATGCGATGGA GAAATGCTCG ATTCAAGATC
551 TGCAAGAGAA ACTTAGCGAC ATTATTATGT AATTGCATGA TGTTCCTCTT
601 TTTTGTPTTT CTAAGACTCC CAGTCAAGAG GAGTATCAAA AGGATTGTTT
651 GTATCAATCA CGATTGAGGT ACTTATTGTT GCTGATGAG TATACATTTGT
701 TATGTAAGAC ATCCACAGAT TTTCAAGAGC AGGCTAGGCC TAAAGAGGAG
751 TTCATTAGGG AGAAATTCAG CCTCTAGAG CTCGAAAGGG GAATAAACA
801 AACTAAAGAG CTTGAGTTTG CATTGCTTAA AAGTAAAGTTA GAACGGGGCT
851 GTTTAGTTAT GAGGAAGTAT GAAGCTGCCG CTAACATAG TTTAGATTCT
901 ATGTTCAAGG AAGAAACTGT GAAGTCGCCG CGGAAGACA CAGAAATA

```

The PSORT algorithm predicts cytoplasm (0.4607).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 116A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 116B) and for FACS analysis.

These experiments show that cp6482 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 117

The following *C.pneumoniae* protein (PID 4376486) was expressed <SEQ ID 233; cp6486>:

```

1 VVVVALFILG IFFLSSSLAF LVHTSCGVLL GAALFILCIG LVLLVALIV
51 FLCHKHKTRQ DLDDYDQQLD SLVIHKKEIP NDISELRFVF EKLNLFPQH
101 TKDFSLSLSE LQKGFNCME KWLTLDEVT KFLIVRDFLL ETRNFTVFG
151 EQVAGIQSNI FDLHEERSSS YLELYRLKRD LQVLLNFLL PPGILKVDYD
201 EIEAIKGLFI RLTSRLDKLD VQAQERKKFI NEMSRFEKVF EKAFIDVDRA
251 TKKLMDRAKK ESPARLFMRG TESLLEMKKN EALNKGGLD FENLSHPELF
301 SPYQQLILN ILNSELVLHH YEFLLSGTIVT SGLTLEECBN RMRRASTGLN

```

351 ALLVRLKQFR GAIKSAYPEK LTIIEKELRS LQDVIKSLER ELIHKIKDIV  
401 TEST\*

The cp6486 nucleotide sequence <SEQ ID 234> is:

5 1 GTGGTGGTTG TCGCTTTATT TATCCTTGGG ATTTTCTTTT TATCTGGTTC  
51 TCTTGCATTC CTGTGTTCATA CGTCTTGGG AGTTCTTTTA GGAGCGCGCG  
101 TTCCCATACT TTGCATAGGT CTGTGTTTAT TGGCTGTAGC TCTTATGTGT  
151 TTCTTATGTCT ACAACACCAA GACTCGTCAA GATTTAGATT ATTTATGATCA  
201 AGATTTTAGAT TCTTTTGGTGA TTCAATAAGAA AGAGATCCCC AATGACATCT  
251 CTGAGTTTGGG GGTAAACATT GAAAAGTTGC AAAATCTGTT TCAGTTCCAT  
10 301 ACGAAAGATT TCTCTGATCT AAGCCAAGAG CTTCAGGGTA AATTTATCAA  
351 TTGCATGGAG AAATGGCTAA CTTTAGAAGA CGAAGTGACT AATTTCTTGA  
401 TTGTTTCGAGA TAGATTTTTTA GAAACCCAGAA GAAATTTTAC CACTTTCTGA  
451 GAACAGGTTA AAGGGATCCA AAGCAATATT TTTGATTTTG ATCAGGAAAA  
501 GTCTTCAATTA TATTTAGAAT TGTATAGGCT TAGGAAGAC CTCCAAGTTC  
15 551 TATTAATAAT TTTTCTGCTC CCCCCAGGTA TACTCAAGGT AGATTATGAT  
601 GAAATTTGAGG CTATCAAAAGG TCTGTTTTATA AGATTAACT CTAGATTAGA  
651 TAAGCTTGAT GTGAAGAGCTC AGGAAGCTAA GAATCTCATT AATGAATTA  
701 GTAGGGAATT TAAAGAAGTA GAGAAAGCTT TGTATATTCT CGATGAGSCA  
751 ACAAAAAAGC TTATGGATAG AGCCAAAGAA GAAAGTCGCG CAGCTCTTTT  
20 801 CATGGGTAGA ACTGAGTCTC TGTGTAAGAT GAAAAAATC GAAGAAGCCC  
851 TTAATAATCA GGGGCTAGAT CTTGAAATC TTTCCATGCC TGAACCTTTT  
901 AGTCCGATAT AACAGCTTTT AATTTGATAT TATTTAATA CGGAATAGT  
951 CTGTCATCAT TATGAGTTCC TTATTTCTGG AACAGTACT TCTGGCCTAA  
1001 CTTCTGAAGA ATGTGAAATC CGAATGAGG CGGCTCTAC TGGTGTGAAC  
25 1051 GCCCTCTTGG TCGCTAGCT CCAATTCAGA GGTGCTATA AATCTCGGTA  
1101 TTTTGAAGAA CTCACAGAGA TTGAAAAGA GTTACAGTCA CTTCAAGACG  
1151 TAATAAAGTC ATTGGAACTA GAATGATCC ATAAGATAAA AGATATAGTG  
1201 ACAGAGAGAA CTTAG

The PSORT algorithm predicts inner membrane (0.7474).

30 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 117A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 117B) and for FACS analysis.

These experiments show that cp6486 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### 35 Example 118

The following *C.pneumoniae* protein (PID 4376526) was expressed <SEQ ID 235; cp6526>:

40 1 MSPFKKIVNR LICYISFOKE SRTLPILIRE PRMTTKSLGS FNSVISGNKI  
51 HFISLGCERN LVDSEVLGI LLKAGYESTN BIEDADYLIL NTCAPLKSR  
101 DEAKDYLDHL IDVKENAKI IVTGCMTSNH KDELPKPMMSH IHYLLSGSDV  
151 ENILSAIESR ESGEKISAKS YBMEGEVFRQ LSTPKHYAVL KVABGCKRRC  
201 APCIIPSIK KLRSKPLDQI LKEFRILVNR SVKEILLIAQ DLG DYKDLIS  
251 TDRSSQLES LHELLKEPGD YWLRMLLYP DEVSDGILL MQSNPKLLPY  
301 VDIPLOHIND RILKQMRRTT SRBQILGFLE KLRKRVQVY IRSSVIVGPF  
351 GETQEBFQEL ADFIGBNWID NLGIFLYSQE ANTPAABLDP QIPEKVKESR  
401 LKILSIQIKR NVDKHNQKLI GEKIEAVIDN YHFEINLLLT ARFYQQAPEV  
45 451 DPCIIVNEAK LVSHFGERC F IEITGTAGVD LVGRVVKKSQ NQALLRTSKA  
501 \*

The cp6526 nucleotide sequence <SEQ ID 236> is:

50 1 ATGAGTCTTT TTAAGAAAAA AGTAAATCCG TTAATATGCT ATATTCTTTT  
51 TCAGAAAAGAA TCAAGAACTC TCCAAATCAT TATTAGAGAA CCTAGGATGA  
101 CACAAAAAAG TTTAGGATCT TTCAATTCAG TTATTTCCAA AAATAAAAT  
151 CATTTTATTA GTTTGGGATG CTTCCGGAAC CTTGTAGAT CGGAAGTCAT  
201 GCTAGGCATT CTTCTTAGGG CAGGTACAGA GTCTACTAAT GAAATTAAG  
251 ATGCTGACTA TTATAATTTA AATACCTGTG CGTTTATAA AACTGCTAGA  
55 301 GATGAAGCTA AAGATTATCT AGACCATCTA ATTGATGTAA AAAAGAGAAA

-145-

351 GCCTAAATTT ATTGTAAC TGATCATGAC TTCCAAACAC AAAGATGAGC  
 401 TTAAACCCCTG GATGTCACAC ATCCATTACC TACTAGGTTC TGGGATGTTG  
 451 GAGAATATTC TTCTGCTAT TGAGTCTCGT GAATCTGGAG AAAAATCTCT  
 501 TCGAAAGAGT TACATTTAGA TGGGAGAGT TCCAAGACAC CTTTCCACAC  
 551 CAAACACCTA TGCCATTATTA AAGTGTGCTG AGGGCTGTAG AAAACGTTGT  
 601 GCTTTTGTGA TTATTCCTTC CATTAAGGA AGGCTCCGCA GCAAACTCTT  
 651 GGATCAAAAT CTTAAAGAA TCCGCATCTC TGTAAACAG AGTGTGAAG  
 701 AGATATATTT GATAGCTCAA GACCTAGGAG ATTAAGGAAA GGATCTCTCT  
 751 ACAGACCGCA GTTCGCGACT AGAATCACTA TTACATGAGT TACTGAAGA  
 801 GCCTGCTGAT TATTTGGCTGC GGATGTTGTA TTTATATCTT GATGAAGTGA  
 851 GTGATGGCAT TATAGATCTT ATGCAACTA ATCCCAACTT TCTCTCCTAT  
 901 GTAGATATTC CTTACAGCA CATTAACGAC CGTATTTTAA AGCAATGGCG  
 951 AAGAAGCACT TCTAGGAGC AATCCTTAGG ATTCCTAGAA AAATACGCTG  
 1001 CCAAGGTTC TACAGTCTAT ATCCGTTCTT CTGTATTTGT GGGTTTCCCC  
 1051 GGTGAAACTC AGGAAGAATT CCAGGAGTGA GCTGATTTTA TTGGTGAGGG  
 1101 TTGGATTGAT AATCTCGGAA TTTTCTTTGA CTCTCAAGAA GCGAATACCC  
 1151 CGGCAGCAGA ACTCCCTGAC CAGATACAG AAAAAGTTAA AGAATCGAGG  
 1201 TTGAAATTC TATCTCAAT TCAGAAACGC AATGTGGATA AACATATCA  
 1251 GAAGCTCATT GGGGAAAAA TAGAAGCAGT TATTGAATAC TATCATCTCT  
 1301 AAACGAATCT TTTATCTACT GCAAGGTTCT ATGGACAAGC TCTTGAAGTG  
 1351 GAACCTTGTG TTATTGTAAA TGAGGCGAAG CTGTGTTCTC ATTTTGGAGA  
 1401 AAGATGCTTT ATAGAAATCA CAGGGAATGC TGGTTACGAC CTGTAGGGC  
 1451 GTGTGTATAA AAAATCTCAG AACCAAGCTT TGCTAAAAAC TAGCAAGCT  
 1501 TAG

25 The PSORT algorithm predicts cytoplasm (0.1296).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 118A) and also as a his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 118B) and for FACS analysis.

30 These experiments show that cp6526 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 119

The following *C. pneumoniae* protein (PID 4376528) was expressed <SEQ ID 237; ep6528>:

35 1 MKNNINNNEC YFKLDSTVDG DLLAANLKTF DTCAQGISST ETFSVQGNAT  
 51 FKQDVSAATGL TSGTTFYNNLA QNFPTSSQIST DFKNNRLSNC ALPKEDCDPV  
 101 PANYVRSPEY FFCSKPLIGD PDPNGBSVL PLTGSSEVLY QSRNVSIFR  
 151 FHWKQSTRE LTVGGRTAIQ FLAAGTYIVS PTVGKRWGNW NGWGAIIYIN  
 201 NGLGQVQCBS TYSGGGYAT IGTLGTSIYR ASVDVAFNPIN DPNASDRYRA  
 251 GIFYLSNGGS SAGIGNYSFS LLTYPPDRG\*

The cp6528 nucleotide sequence <SEQ ID 238> is:

40 1 ATGAAAAACA ATATTAAATTA TAATGAGTGC TATTTTAAAT TAGACTCAAC  
 51 TGTAGATGGT GATTGTGTAG CAGCCAATCT CAGACCTTTT GATACACAGG  
 101 OCCAAGGAAT CTCATCGACT GAAACATTTT CTGTTCAAGG GAATGCAACA  
 151 TTTAAAGATC AAGTTTCAGC AACTGGATTA ACTTCAGGAA CTACTATATA  
 201 TTTAAATGCA CAAAACTTTA CTCTCTCCCA AATCTCTATA GATTTTAAAA  
 251 ATAAATCGCT GAGTAATTTG GCATTTGCCA AAGAAGACTT CGATCCGGTG  
 301 CCAGCGAATT ATGTTCTGTTT TCCCGAATAT TTTTCTGTGT CCAAGCCTCT  
 351 GATCGAGAGT TTGATTTTAA ACTTCAGGGA ATCTATTTTG CCTCTGACTG  
 401 GTTCGGAATA TACTCTATAT CAGTCACGTA ATGTAAATAG TATATTTGCT  
 451 TTTATAGGAT GGAAGCAAGT TACACGAGAA TTAACGTAGT GGGGAATATC  
 501 TGCAGATCAA TTTCTTGCA GAGGAACCTA TATCGTTTCA TTTACTGTGT  
 551 GTAAACGGTG GGGATGGAA TATGTTTGGG GAGGAGCCAT TTATATCAAT  
 601 AATGGTTTAG GACAAGTCCA ATGTGAAAGC ACGAATTTATA GTGGTGAGG  
 651 GTATGCAACA ATAGGTACAC TGGGGACCTC AATATATAGA GCCTCTGTAG  
 701 ATGTAGCTCC TAATCTTAAT GATCCGAATG CTTCGGATCG CTAATAGAGC  
 751 GGTATTTTCT ATCTCAGTAA CGGTGTTTCT AGTGCAGGTA TAGGGAATTA  
 801 CTCTTTTCTT CTCTCTAAT ATCCGACAGA TAGAGGGTAG

The PSORT algorithm predicts cytoplasm (0.1668).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 119A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 119B) and for FACS analysis.

- 5 These experiments show that cp6528 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 120

The following *C.pneumoniae* protein (PID 4376627) was expressed <SEQ ID 239; cp6627>:

```

10      1 MKCSPLTLVP HIFLNDCBC HRSCSLKIRT IARLILGLVL ALVSALSFVW
      51 LAAPISYAIG GTLALAAIVI LIITLVVAIL AKSKVLPIN ELQKIYNRY
101    PKEVFYFVKT HSLTVNELKI FINCWKSGTD LPPNLHKRAE AFGIDILKSI
151    DLTLFFPEFEE ILIQNCPLYW LSHFIDKTES VAGBIGLNKT QKVYGLLGLPL
201    AFHKGVTITF HSYTRPLILT LSESQYKPLY SKASKNGWDS PSVKKTCEBI
251    PKELPHNMF RKDVQISQF LFLFFSHGIT WQQAQMIQLI NPDNWMKLCQ
15     301 FDKAGGHCSM ATFGGFLNTE TNMPDPVSSN YEPTVNFMTW KELKVLEKVV
351    KESPMHPASA LVQKICVNTT HEQNLILKRWQ FVRNTSSQWT SSLPYAFPHA
401    QTYKLEKKIE SSLPIRSSL*
```

The cp6627 nucleotide sequence <SEQ ID 240> is:

```

20      1 ATGAAGTGTA GTCTTTAAAC ACTAGTTCCT CATATATTTT TAAAAAATGA
      51 CTGCGAATGTT CATAGATCTT GTCTTTTAAA AATTAGGACA ATTGCCCGAC
101    TCATTCCTTGG GCTTGTTCTA GCTCTTGTTA GCGCACTTTC TTTTGTTTTC
151    CTTGCTGCGC CGATTAGCTA TGCTATTGGA GGAACTTTAG CTTTAGCCGC
201    TATCGCTAATC TTGATTATAA CGCTAGTCGT AGCACTGCTA GCTAAATCAA
25     251 AGGTTCCTGCC CATCCCCAAC GAACTTCAGA AGATTATTTA CAATCGCTAT
301    CCTAAGAAGG TCTTTTATTT CGTGAAACAA CACTCCCTGA CTGTAAACGA
25     351 ATTAAAAATA TTTATTAAIT GCTGGAAGAG CGGTACAGAC CTGCCCTCCA
401    ATTACATATA AAAAGCAGAG GCTTTCGGGA TCGATATTCT AAAATCTATA
451    GATTTAACCC TGTTTCCAGA TTTCGAGAG ATTCTTCTTC AAAACTGCCC
501    GTTATACTGG CTCFCCCAT TTTATAGACAA AACTGAATCT GTTGCTGGGG
30     551 AAATCGGATT AAATAAAACA CAATAAGTTT ATGGTTTACT TGGGCCCTTA
601    GCGTTTCATA AAGGATATAC AACTATTTTC CACTCTTATA CACGCCCTCT
651    ACTAACATTA ATCTCAGAA TACAGATATA GTTCTATAT AGTAAAGCGT
701    CTAAGAATCA ATGGGATPCT CCTTCTGTGA AAAAAACCTG CGAAGAAATA
35     751 TTCAGGAAC TCCCCCAACA TATGATTTTC CGGAAGGATG TTCAGGAAT
801    CTCACAAATC TATTTCCTTT TCTTTTCTCA TGGTATCACT TGGGAACAGG
851    CTCAGATGAT TCAACTTATA ATCTCTGATA ATTGGAATAT GTTGCTGACG
901    TTTGATAAAG CAGGAGGCCA CTGTTCCATG GCAACATTTG GAGGCTTTT
951    GAATACTGAA ACAAAATATG TCGATCCAGT ATCCTCTAAC TATGAACCTA
1001   CAGTGAACCT CATGACGCTG AAGAAATTTA AGSTTTTACT AGAGAAAGTA
40     1051 AAAGAAAGTC CTATGCACCC AGCGAGTGCT CTTGTTTCAA AGATATGCGT
1101   AAATAACAAC CACCATCAAA ATCTGTGTA ACGATGGCAA TTTGTTGCTA
1151   ATACGAGTTC ACAATGGACA TCAAGCTTAC CTCAGTATGC TTTCCACGCC
1201   CAACCTACA AACTAGAGAA AAAAAATAGAA AGCAGTCTCC CTATACGATC
1251   TTCCTATAA
```

- 45 The PSORT algorithm predicts inner membrane (0.7198).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 120A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 120B) and for FACS analysis.

- 50 These experiments show that cp6627 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.



**Example 121**

The following *C.pneumoniae* protein (PID 4376629) was expressed <SEQ ID 241; cp6629>:

```

1 MSNITSPVIO NNRSCNYFE LKNSTTHIV I SAILLCGAL IAFLCVAAPV
5 51 SYILSGALLG LGLLLIALIGV ILGIKKITPM ISSKEQVFQ ELVNRIRAHY
101 PKFVSDFVSE AKPNLKLDS FIDLLNQLHS EVGSSNMYNV SEELQOKIDT
151 PEGIARLKNE VRTASLKRLE SAASSRPLFP LSPKILQKVF PFWLGEFIS
201 AGSKVVELHR VKKIGSLBE DLSDYIKPEM LPTYWLIPLD FRPTNSSLIN
251 LHTLVLARVL TRDVFQHLKY AALNGEWNLN HSDLNTMKQVQ LPAKYHAAYQ
301 SYKHLSPQSL OEDEFYNLLL CIPKHYISWK QMSLKTQVPA DLWNLCCLT
10 351 LDHTGRPQDM EFASLIQTLT TQGLIHKESE AFLSSLTLLS LDQFKTIRKQ
401 STNIAMFLEN LATHNSTFRS LPFITVHPLK RSVFSQPEED BSSLIG*
```

The cp6629 nucleotide sequence <SEQ ID 242> is:

```

1 ATGAGTAATA TAACCTCGCC AGTTATTCAA AATAATCGCT CTGTAAITTA
5 51 TTAATTTTGA TTAAGAATT CAACCACTAT TCATATTGTT ATCAGTGCCA
101 TCTTACTCTG CGAGCTTTG ATAGCTTTCT TGTGTGTAGC AGCTCTGPTT
151 TCCTATATTC TAGTGGCSC ATTGTTAGGA TTAGGAATAT TAAAGCCTT
201 GATTGTGTG ATTTAGGAA TAAAAAAAT CACGCCTATG ATTTCACTAA
251 AAGAACAAGT ATTCCCCCAA CACTCGTAA ATAGARTCAG GGCGCACTAT
301 CTTAAATTTG TCTCTGATTT TGTTCAGAA GCTAAACCAA ATCTTAAAGA
20 351 TCTCATAAGT TTAATGATC TTTCAATCA ATTGCACCTC GAAGTGTGAT
401 CATCTACAAA TTACAGCTA TCTGAAGAAC TACAACAGAA AATAGATACG
451 TTCGAGGGTA TCGCACGCTT AAAAAATGAA GTCCGTAAGT CTTCCTCTAA
501 AAGACTTGAA AGCGCTGCTT CTTCCTGCTC CTCTCTCCCC TCTTTACCAA
551 AAATCTTACA AAGGTATTT CCATTTTCTT GGTTAGGAGA GTTTATTTCT
25 601 GCAGGCAGCA AGGTTGTAGA GCTCCATCGA GTTAGAGAAA TTGGAGGCAG
651 CCTCGAAGAA GACCTTAGTG ATTATATAAA ACCAGAGATG CTCTCTACCT
701 ATTGGTGTAT TCTCTTAGAT TTTAGACCAA CAAATTCCTC TATTCTAAAT
751 CTACACACAC TAGTTTAGC TAGAGCTCTA ACTCGTAGTG TTTTCTAACAA
801 TCTTAAGTAT CGAGCAATTA ATGGCAGTGT GAACCTGAAAT CATAGTGATC
30 851 TAAATACTAT GAAACAGCAG CTCTTTGCTA AATATCATGC GGCGTATCAA
901 TCCTATAAAC ATCTATCTCA ACCCTCTCTT CAAGAGGATG AATTCTATAA
951 CCGTCTCTTG TGTATTTTTA AGCATAGGTA CTCGTGGAAG CAGATGTCTC
1001 TAAATAAAAC AGTCCCGGCT GATTATTGGG AAAACCTCTG TTGCTTGACT
1051 TTAGACCATA CAGGACGACC CCAAGACATG GAAITTCGCT CTCTAATTGG
35 1101 TACTCTCTAC ACACAAGGCC TAAITTCATA AGAAAGCGAA GCATTTCTTT
1151 CTTCATTGAC ATCTCTTAGT TTAGATCAGT TTAAAGCAGT CCGTCGTGAC
1201 TCAACCAATA TAGCAGTGT CTTGAGAAAT TTAGCAACTC ATAAATCCAC
1251 CTTTAGAAGC TTACCACCTA TRACAGTCCA TCCACTCAAG AGAAGCGCTC
1301 TCTCCCAACC TGAAGAAGAC GAGTCCCTCC TGCTSATAGG TTAG
```

40 The PSORT algorithm predicts inner membrane (0.5776).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 121A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 121B) and for FACS analysis.

These experiments show that ep6629 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

**Example 122**

The following *C.pneumoniae* protein (PID 4376732) was expressed <SEQ ID 243; cp6732>:

```

1 MEMMSPFQQP BQCHFDVVGS FLRPESLTRA RSDPFEBGRIV YBQMRVVEDA
5 51 ATRNLKKQT EAGLIFPTDG EFRYSWDFD FMWGFHVDNR RRDNDPEIG
101 VYLKDKISVS KHPFIEHFEF VKTPEKGNAX AKQITLPSPSQ FFHEMIFAPN
151 LKNTREFYPT NQELIDDIIVE YVRQVIQDLY AAGCRNLQLD DCACRLLDID
201 RAPSWGYGVS HDRLQELIQE FLWIHNLVWK DRPDLFVSL HVCRRGDYAE
251 PFSRRAYDSI BEPLFAKTDV DSYHYWALD DKYSGGAEPF AYVSGEKKVC
301 LGLISSNHSC IEDRDVVSR IYEAASYIPL ERLSLSPQCG FASCBGDRHM
```

351 TEEEQMKLIA PVKEIAKEIW G\*

The cp6732 nucleotide sequence <SEQ ID 244> is:

5 1 ATGGAATGA TGAGCCCAT CCAACAACCT GAGCAATGTC ATTTTGATGT  
51 TGTGGGAAGT TCTTACGTC CTGAAAGTCT TACACGAGCA CGCTCTGATT  
101 TTGAAGAAGG AAGAATTGTC TATGAGCAGA TCGAGATGCT CGAAGATGCT  
151 GCTATTGCTA ATCTCATAAA AAGGCAAAACA GAAGCAGGTC TTATCTTTTT  
201 TACTGATGGG GAATCCCGTA GGTATAGTTG GGAATTCGAC TTATATGTTGG  
251 GATTCCATGG CGTGGATCGT CGCAGGGACT CTAATGACCC TGAAATTGGG  
301 GTGTATCTTA AAGATAAAAT CTCGATATCA AAACATCCGT TTATAGAACA  
351 TTTGAGTTT CTGAAACTT TTGAGAGGG AAATGCAAAA GCAAAACAAA  
401 CGATTCCTTC TCCATCACA TTTTTCACAT AGATGATTTT TGCTCTTAAT  
451 CTGAAAAATA CTGGGAAGTT TTATCCTACG AATCAAGAGC TAATTGATGA  
501 TATTGTCTTT TATTATCGCT AAGTCAATCCA AGATCTTTAT GCTGCAAGTT  
551 GTCTGTAATT GCAGTTGGAC GATTGTGCTT GGTGTGCGCT CTGAGATATA  
601 CGAGCGCCTT CTTGGTATGG TGTGTATTCT CATGACAGGT TGCAGGAAAT  
651 TTTAGAACAG TTTTATGGA TCCATAATT AGTGATGAAG GATAGACCGG  
701 AGGATCTTTT TGTATGCTG CATGCTGTCT GTGGTGATTA TCAGGCCGAG  
751 TTTTCTCTTA GACGAGCTTA TGATCTATA GAGGAGCCTT TATTTGCTAA  
801 GACCGATGTG GATGATATCT ACTATTATAT GCGCTCTTAT GATAAGTATT  
851 CAGGAGGTGC TGGCCCTTTA GCTTACGTC CTGGAGAGAA ACACGCTGCG  
901 TTGGGATGTA TCTCCAGCAA CATCTCTTGT ATTGAAGATC GAGATGCTGT  
951 GGTTCCTCGT AATTATGAAG CTGCGAGCTA CATCTCCCTA GAGAGACTTT  
1001 CTTTGAGCCC GCAATGTGGG TTGTGTTCTT GTGAGGAGGA CCATAGAATG  
1051 ACTGAAGAG AACAGTGGAA GAAGATCGCC TTTGTGAAG AGATTGCTAA  
1101 AGAGATCTGG GGAATA

The PSORT algorithm predicts cytoplasm (0.2196).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 122A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 122B) and for FACS analysis.

30 These experiments show that cp6732 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 123

The following *C. pneumoniae* protein (PID 4376738) was expressed <SEQ ID 245; cp6738>:

35 1 VMLRFLILVS YDEKEKDVVV VCNHSEPNIL GLPPEAVSQI IEELSDBGYS  
51 YLNVVRCDLS GETTVQORLL LNADEGRSMT VVISELPEGH PDIRINQLAS  
101 ERIFVSRKEE AADAYASGCK VVAFDDEHLP WVSSHIAVE EIREKQBQTM  
151 QGSLITEQLG ALLCNIVSTE KNLAFALDAV IKQSVWFRFN PDLFAVEREA  
201 LEASVTDALV SYVSNLMDIP YTSQGVIVIE DSSIVRTSQE HTLIVNCAAF  
251 DKLASQIEFL CPSDLVPISG KDFLISDDED EELNPKVSSA ADSKDKT\*

40 The cp6738 nucleotide sequence <SEQ ID 246> is:

1 GTGTGGCTGC GCTTTTTACT TTTAGTGTCC TATGATGAGA AGGAGAAAAG  
51 CGTAGTGTGC GTTTGTAATC ATTCTGAACC TAAATATCCTC GGCTGCTCTC  
101 CTGAAGCAGT CTCTCAGCTT ATTGAAGAGC TTAGCGATGA AGGCTATAGC  
151 TATCTGAATG TAGTGCCTTG TGAATCTCTC GGGGAGACTA CGGTTCAACA  
201 ACGTCTGCTA TGAAGTCCG ATGAAGGGAG ATCTATGACG GTGGTGATCT  
251 CAGAGCTTCC TGAAGGGCAC CCGAATATTC GGAATTGACA GTTGGCATCC  
301 GAAAGAAATT TTGTTCTCTG TCGAATAAGAA GCTGCTGATG CTTATGCTTC  
351 AGGATGTAAA GTGGTGCTCT GACATCTCCC TGSGTCTCACA  
401 GTCATATTGC CTACGCGGAG GAGATCAGAG AGAAACAAGT ACAACAAGT  
451 CAGGGGTCTT TAACGTGAAG GCAGTGTAGA GCACCTCTCT GCACACAAGT  
501 CTCCACAGAG AAAAATCTAG CCTTGTGCTT AGACGCCGCTG ATAAACAAGT  
551 CTGTGTGAG ATCTCGCAAT CCGGATCTTT TTGCTTAAGA GAGAGAAGCT  
601 CTAGAGGCTT CAGTAAACAG TGCCTTAAGA TCTTACGTGT CAATTTAGA  
651 CATGATACCG TACACAAGTT CTACGAGCAT AGTCATAGAA GATAGTAGTA  
701 TCGTCCGTAC CTCTCAAGAG CATCACTCA TTTGGAATG TGCAGCATTC

```

751 GATAAGTTAG CGAGCCAAAT AGAGTTCTTA TGCCCCAGTG ACGTGTGCC
801 CATTTCTGOT AAGACCCCTT TGATTCTCGA TGAAGGAT GAGGAACCTGA
851 ATCCTAAAGT TTCATCTGCT GCAGACTCTA AAGATAAAAC CTAG

```

The PSORT algorithm predicts cytoplasm (0.1587).

- 5 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 123A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 123B) and for FACS analysis.

These experiments show that cp6738 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

## 10 Example 124

The following *C.pneumoniae* protein (PID 4376739) was expressed <SEQ ID 247; cp6739>:

```

1 MTHCLHGWFS VVRHHFVQAF NFRSPLYSRI THFALGVKA IPIVGHVWG
51 VDWLILSHCFE RGVSHPGFPS DIAPILKVEK IAGRDHISRI ENQLKSLRKT
101 IEVEDLDKVH GQYQENPYAD MASSEVLKLD KGVHVSSELG AFSRVRNRIT
151 RSYSYAPTPO LDSTAIIVGID LVSPBEOENL VRLANEVLOL YPKSKITLYL
201 LIDFNKEWVG DISSDKKKQL RSLGLHSEVQ CLSVLEPQGA EGEDTKHPDL
251 MVGCGYKDSY LRSKILQQA LGTSLGTVPW VNVHMLPFR YRSRLSLPIN
301 TEKDKTELYK EISRTHQLH TLMGLGAQD SGLLLDRQLR HAPLSQGSCH
351 HSYLADLTHE ELKILLPSAF VDAKNISKRE LREVSILNFA DTSVECGCAF
401 YF*

```

The cp6739 nucleotide sequence <SEQ ID 248> is:

```

1 ATGACTCATT GCTTACATGG TTGGTTTTCT GTAGTTCTGT ATCACTTTGT
51 GCAGGCGTPT AATTCTTCAC GTCCTTTATA TTCTCGAANT ACCCACTTCG
101 CTTTAGGGGT GATTAAAGCC ATCCCATTTG TAGGGCATCT TGTATAGGGA
151 GTCGAFTGGT TGATCTCTCA TTGCTTCGAG AGGGGAGTCT CACACCCGG
201 GTTCCCTTCA GATATTGCTC CTATACTGAA AGTAGAAAG ATCCGGGGCC
251 GAGATCATAT TTCTAGAAAT GAAAATCAGC TAAAGAGCCT TAGGAAACT
301 ATCGAGGTGG AAGATCTAGA TAAAGTCCAC GGCAATATC AAGAGAATCC
351 TTATGTCAGAT ATGGCCTCTA GTGAGGTCTT TAACTCGAT AAGGGAGTTC
401 ATGTTAGCGA GCTTTGCCAA GCCTTTTCTA GAGTTTCGAA TCCTCATACC
451 AGATCCCTATA GTTATGCCCC TACTCCTCAG TTGACACTTA TAGCTATTGT
501 TGGTATAGAT CTCTCAAGTC CTGAAGAACA AGAGAATTTA GTACGCTTGG
551 CGAATGAGGT CATTCACACT TATCCCAAA CAAAGACAAC TCTATATCTT
601 CTTATCGATT TTAATAAGGA GTGGGTAGGG GATATCTCCT CTGATAAGGA
35 651 AAAACAGCTC CGTTCTCTAG GTCTACATTC TGAAGTTCAG TGCTTTCCCG
701 TCTTGGAAACC TCAGGGTGCC GAGGGCGAAG ATACGAACA CTTTGACCTT
751 ATGGTCCGCT GTTATGGGAA GGAATCTTAC TTAAGGGAGG GTAAATTTT
801 ACAGCAGGCC CTAGGGACTT CGTTAGGTAC TGTTCCCTGG GTGAATGTTA
851 TGCACACATP GCCATCTAGG TATAGATCTC GGCCTTCCTT ACCATATAAT
40 901 ACCGAAAGGG ATAAGACAGA GCTTTATAAA GAGATTTCTC GTACACACCA
951 TCAGTTGCAT ACTTTGGGAA TGGGACTTGG AGCCGAGGAT TCAGGATTCG
1001 TCTTAGACCG GCAACGACTC CATGCTCCTT TATCTCAAGG GTCTCACTGC
1051 CATTCCTCAT TTGCGAATCT CACCACATGA GAGCTGAAAA TTTTGTATT
1101 TTCAGCATTT GTGATGCTA AGAACATAAG TAAGAAGAGG CTTCTGTAGG
45 1151 TATCTCTAAA TTITGCTAAC GATACTTCGG TAGAGTGTGG CTGCGCTTTT
1201 TACTTTTAG

```

The PSORT algorithm predicts inner membrane (0.2190).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 124A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 124B) and for FACS analysis.

These experiments show that cp6739 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 125

The following *C.pneumoniae* protein (PID 4376741) was expressed <SEQ ID 249; cp6741>:

```

5      1  MASCLSAWFS  IVREHFYRAF  DFLSLPFCARI  TEFVLGVIRG  IPVVGHTIIVG
51     51  IEWLSRYLE  SFVTKPTFVS  DVVSLKTEK  VAGRDHIARV  VETLKQRQVA
101    101  VAPDEDEKVH  GKIPVHPFGS  TQFVENVLTY  FEVQDATLGL  AFSKIRNRVR
151    151  QAYLQAPRPK  LQKIYIIGND  MNPFVDDFL  HLARLCNEVQ  RLYPDATISL
201    201  YLTASGGRNA  MDDKNRKLIS  DCELNPKIAC  LDNPQGDVVK  QATCDCEWVY
10     251  HGENDQGTIN  QIQBELEKSG  BETPMIHVGQ  KPLSQSLWDF  SPFSSLEMMK
301    301  DKEKALEYSE  LEKEQLYSRL  VYVGERSSVL  SLPGDSSRSQ  ILMDPKRVHA
351    351  PLESEHYCHS  YLADLENPGL  QKTILAFLN  PKELSSITLQ  PISLNLILNS
401    401  KTYLRQHFGF  FERMSKSDRN  VVVVCDSDW  GTDKWKEPSF  QHFTMELBCR
451    451  GYSHFNIAF  RSNMCEVEER  RILNESSQEK  APTMIFCEDS  VSQGDIRCLH
15     501  LASEGMLCGK  BCYAVDVYTS  GCANFMMKEV  L/LERESNLN  NRKHGLWKRRE
551    551  VRKQKEAAL  DQDESEIYVC  NQLTAQQNFA  CS*

```

The cp6741 nucleotide sequence <SEQ ID 250> is:

```

1      1  ATGGCTCTCT  GTTATCTGCG  CTGGTTTTCT  ATAGTTCGTG  AGCACTTTIA
51     51  TCGAGCCTTT  GATTTTCTCT  TGCCGTTTTG  TGCTCGTATT  ACGGAATTGG
101    101  TATTAGGGGT  CATCAAGGGG  ATCCCTGTTG  TGGGTCACAT  TATTGTTGGG
151    151  ATAGATGGC  TCGTTCTTAG  GTATTTAGAG  AGTTTCTGTG  CCAAGCCGAC
201    201  ATTTGTCCT  GATGTGGTGA  GTCTTCTGAA  AACAGAGAAA  GTTGTGGTCT
251    251  CGCATCACAT  TGCTCGTGTG  GTGGAGACTT  TGAAGAGGCA  GAGAGTCGCT
301    301  GTGGCTCCTG  AAGTAGAGGA  TAAGGTCCAT  GGGAGATPCT  CTGTGCATCC
25     351  TTTCCGGGGA  ATCCAACTGC  TAGAAGTCTC  CACTCTCTAT  CCGGAAGTTC
401    401  AAGATGCAAC  GTTAGGGCTT  GCCTTCTCTA  AAATTCGTAA  TCGTGTAAAG
451    451  CAGGCGTATT  TGCAAGCTCC  ACGGCCAAAA  CTGAGAAGA  TTTACATCAT
501    501  AGGAAACGAT  ATGAATCTCT  TTGAAGTTGA  CGACTTCCTG  CATCTAGCCC
551    551  GTCCTGTGTA  TGAAGCTCAA  AGACTCTATC  CTGACGCTAC  GATTTCTCTA
30     601  TATCTAACAG  CTTCTGGTGG  TCGCAAATGT  ATGACAAAA  AGAATCGGAA
651    651  GTTACTTAGT  GATTGCGAAG  TAAACCCCAA  GATTCCTTGT  TTGGACTTTA
701    701  ATCAGGGTGA  TGTATGCAAA  CAAGCAACTT  GTGACTGTGT  GATGGTGTAT
751    751  CATGGGGAGA  ATGATCAAGG  TACGTTGAAT  CAGATTGAG  AAGAGTTAGA
801    801  AAAGTCAGGG  GAGGAAACCC  TCTGGATTCA  TGTGGGGCAA  AAGCCTCTTT
35     851  CACAACTCTT  GTGGGATTTC  TCTCCATTTT  CATCTTTTGA  GATGAAGGGA
901    901  GATAAAGAGA  AAGCTCTAGA  GTACTCTGAA  TTAGAAAAAG  AAGCACTATA
951    951  TTCTCGATTG  GTATACGTAG  GAGAGCGCTC  TTGGTTTCTT  AGTTTGGGGT
1001   1001  TTGAGAGTAG  TCGGTCAAGG  ATCTTGATGG  ACCCAAAACG  TGGTCATGCT
1051   1051  CCGTTATCTG  AAGGGCATTG  TTGTCAITCC  TACCTTGCAG  ACTTAGAAAA
40     1101  TCCCGGGTTA  CAAAAACAA  TTTTAGCGCG  ATTCTTGAAT  CCTAAGGAAT
1151   1151  TGAGCAGTAG  CACTACTGCA  CCTATACTC  TAAATCTTAT  CTTAAATAGC
1201   1201  AAAACTTACT  TAAGGCAGCA  CTTTGGCTTT  TTGAGAGGGA  TGAGCAGAGA
1251   1251  TGATCGCAAT  GTGGTTGTGG  TTGTATGTGA  TTCTTGTGTG  GGTACCGACT
1301   1301  GGAAGGAGGA  GCCAAGCTTC  CAACACTTTA  TTATGGAGCT  AGAGTGTGCA
45     1351  GGGTATTTCG  ACTTCAATAT  TTTTGCCTTT  AGATCTAATA  GCATGTGTGT
1401   1401  AGAAGAACGT  AGGACTCTTA  ATGAAAGTTC  TCAAGAGAAA  GCCTTTACCA
1451   1451  TGATTTTCTG  TGAGGATTCA  GTATCTCAAG  GAGATATCCG  CTGTTTGCAAT
50     1501  TTGGCGTCTG  AAGGAATGCT  TTGTGGTAAA  GAGTGGCTATG  CTGTCTGATG
1551   1551  CTATACGTCA  GAATGCGCGA  ACTTTATGAT  GGAAGAAAGT  TTAACCTTGG
1601   1601  AGCGAGAAAT  TAATCTGTGG  AATAGAAAGC  ATGTGCTTTG  GAAAAGAGAA
1651   1651  GTTAGAAATC  AGAAACAAGA  AGCTGCTTTG  GTACAGACAG  AGAGCGAGAT
1701   1701  TTACGTTTGT  AATCAGCTGA  CGGCGCAACA  GAATCTGCTC  TGTCTTTGA

```

The PSORT algorithm predicts inner membrane (0.2869).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 125A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 125B) and for FACS analysis.

These experiments show that cp6741 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 126

The following *C.pneumoniae* protein (PID 4376742) was expressed <SEQ ID 251; cp6742>:

|    |     |             |             |             |             |             |
|----|-----|-------------|-------------|-------------|-------------|-------------|
| 5  | 1   | LFVSPFIPIFV | VMPFIPISSW  | ISTVROHFVK  | AFDPSRPFGS  | RVTNPFALGVI |
|    | 51  | KAIPVIGHIV  | MGMEWLVSSE  | VAGLITRSSP  | TSDEVQIVKT  | EKALGRUHS   |
|    | 101 | RVAEILLQREK | GTITFENGDK  | VHGKFPVCPF  | GRKLSSEBTHK | LKPSREGTL   |
|    | 151 | DVPSFVIRK   | VTRAYLQAPR  | FEIRFISIVG  | SKLKTQDPF   | QVSLANETIQ  |
| 10 | 201 | RLHPEALVCR  | YLGTGLNRESQ | NCITPTTARKK | QYLHNSGLDS  | RQCKDSKED   |
|    | 251 | DAGSPENPEL  | WIGYSREQQ   | HNLDQYVQQ   | CLGKSDAPFP  | WLVVIEDTKD  |
|    | 301 | FYFPPNPFSTY | LMDPTQSTDP  | SPRPLPESSE  | DKDLSLYGLS  | RYHYHEMLG   |
|    | 351 | LGLKPEDAGL  | LMDPRIIYAP  | LSQGHCHSEY  | LADIENEDLR  | TLVLSPLFLP  |
|    | 401 | QNLSSDELRP  | VAFNIALRPL  | ELDSLFRNIV  | AQQQGRNIV   | TLAHGTPRE   |
|    | 451 | DLDPDPMNIL  | TKRLQMSQYS  | VLNIFSVKSR  | KMIVKERQPF  | GDREBGSST   |
| 15 | 501 | LILFEDPISA  | ADFRCLQIAA  | BGMVAKDLES  | VADICASGCS  | CIOFSEMOSSP |
|    | 551 | QAIKEYRONEA | RVEDAAGEEA  | REPIVYSQDP  | LSSMLTQCN   | FVPSLDAAVVK |
|    | 601 | QAIWRFRSKG  | LITMERKALG  | REPLTAIFYSY | LGSQERNERM  | GKRTTEEHEV  |
|    | 651 | VISPEELDRM  | VQVLPAAEVA  | DSGNDPTRPV  | PNPDSNPDS   | QNEGS*      |

The cp6742 nucleotide sequence <SEQ ID 252> is:

|    |      |             |             |             |             |             |
|----|------|-------------|-------------|-------------|-------------|-------------|
| 20 | 1    | TTGTTTGTTT  | CTAATTTTAT  | TTTTTTTGTT  | GTATATGCCAA | TTCCCTATAT  |
|    | 51   | TTCTCTCTGG  | ATTCTCTACG  | TTCCACACGA  | TTTTGTTTAAG | GGGTTTGATT  |
|    | 101  | TCTCTCTGCC  | CTTTTGTCTT  | AGGGTTACGA  | ATTTTGTCTTT | AGGGGTCTAT  |
|    | 151  | AAGGCATCTC  | CTATTGTAGG  | ACATATTGTC  | ATGGGGATGG  | AGTGTGTIAT  |
| 25 | 201  | TTCTTCTCTG  | GTTGCCGGGA  | TTATTACTAG  | GCTCTCCTTT  | ACCTCAGATG  |
|    | 251  | TCGTTTCAGT  | TGTAAAGACT  | GAGAAGCGGT  | TAGGTCTGAGA | TCATATATCT  |
|    | 301  | CGAGTGGCGG  | AGATATTGCA  | AAGAAGAAAG  | GGGACCATAA  | CTCCTGAGAA  |
|    | 351  | TCAAGATAAG  | GTGCAATGGG  | AGTTTCTCTG  | CTGTCTCTTT  | GGTCTGTTAA  |
|    | 401  | AATCCGAGGA  | AACCTTAAAA  | CTTAAGCCGG  | GAGAAGAGAA  | GGGAACCTTA  |
| 30 | 451  | GATACTGTAT  | TTTCTCGATG  | TGCGACCGCG  | GTGACTCTGT  | GCTACTTACA  |
|    | 501  | GGCCCCCGGA  | CCCGAAATAC  | GTACGATTTT  | TATTGTGGGT  | TGAAACCTTA  |
|    | 551  | AAACTCTCTA  | AGATTTCTCG  | CAATTGTGGA  | GTCTCGCGAA  | TGAAACGCGA  |
|    | 601  | AGACTGCATC  | CTGAAGCGTT  | AGTTTGTCTG  | TATTTTGACAG | GCTTGAATCG  |
|    | 651  | CGAATCTCAG  | ATGTGCGATA  | CAACTACTGC  | AGAGAAGAAG  | CAGTACCTAC  |
| 35 | 701  | ATAACTCAGG  | TCTCGACTCT  | AGAACTCAGT  | GCAAGACAGC  | TAAAGAAAGC  |
|    | 751  | GACGCTGGCT  | CTCCGAAAAA  | TCCCGAACTT  | TGGACTGGCT  | ATTATTCACG  |
|    | 801  | AGAGCAACAG  | CATAATATAG  | ACGGCGAGTA  | TATTTACAGC  | TGTCTAGGGA  |
|    | 851  | AGAGTCACAG  | TCCAATCTCT  | TGSAATCTAT  | TACTTGAGCA  | CAAAAGAGAT  |
|    | 901  | TTTTATACCC  | CACCAAACTT  | TACTTCTCAT  | TCACATACAA  | GACAACTCAT  |
|    | 951  | AGACCCACAC  | TCCGCAACCA  | GACTCCCTGA  | AAGTGAGGGG  | GAAATCGGAT  |
| 40 | 1001 | CGTCTGTACG  | ACAACTGAGT  | CGATCTGTAT  | ACCATGAGTA  | TATGCTTGCT  |
|    | 1051 | TTGGGATATTA | AACTCAGAGG  | TCACGACACT  | CTGATGGACC  | CGGATAGAA   |
|    | 1101 | CTATGCTCTCT | TATCTCCACG  | GCACATATGT  | TCATCTCATC  | CTTGCTGGTA  |
|    | 1151 | TAGAAAAATGA | GGATCTACGA  | ACTTTAGTCC  | TTTTCGCTCT  | CTTAGATCTT  |
|    | 1201 | GGCAATCTTA  | GTAGCGAGGA  | TCTTCTCTCT  | GTAGCAATCA  | ATATCTCTAG  |
| 45 | 1251 | ATTGCCATTA  | GAATTCGACT  | CGTATATTTT  | CGGCTGTGTG  | GGGGGTCAGC  |
|    | 1301 | AAGAAAGGAG  | AAACATAGTT  | ACCTTTGCCG  | ACGGAATCTC  | TCGTCAGAGA  |
|    | 1351 | GATCTTGAATC | CTGACTCAAT  | GAACATCTCG  | ACGAGAAGAT  | TACAAATGTC  |
|    | 1401 | TGATATATAG  | TATTTGAACA  | TTTCTCTCTA  | TAAATCAAGG  | AAATGATTGT  |
|    | 1451 | TAAAGAAAGC  | TCAGTCTCTT  | GGAGATCGTT  | CTGAAGGAGAA | GTCTTTTCACA |
| 50 | 1501 | TTGATCTCTAT | TGTAGGATCC  | CATTAGTGCA  | CGAGATTTCC  | GTGTTTTCGA  |
|    | 1551 | GCTAGCTGCA  | GAGGCTATGG  | TTGCTAAGGA  | TCTTCCCGAGC | GTAGCAGTCA  |
|    | 1601 | TTTGTGGCTTC | TGATGTTTCC  | TGCAATTCAGT | TTTCTGAGAT  | CGAGATCTCT  |
|    | 1651 | CAGGCTATTTG | AATATAGACA  | ATGGGAGGCA  | CGTGTGCAAG  | ATTGAAGCAGG |
|    | 1701 | AGAAGAAGCC  | AGAGAACCAG  | TAATTTATTC  | TACAGGATCAA | TTGAGCAGCA  |
| 55 | 1751 | TGCTTCACTAC | ACAAACGAAT  | TTTGTATTPT  | CTCTAGATGC  | TGTGGTAAAA  |
|    | 1801 | CAGCGGATCT  | GGAGATTCGG  | TTGCAAAAGGT | CTTCTTACTA  | TGGAAAGAAA  |
|    | 1851 | GGCACTAGGC  | GAGGATTTCT  | TAACTCGGAT  | ATTTTCTCAT  | TTAGGAGGTC  |
|    | 1901 | AGGAGCGTAA  | TGAGAATATG  | GGGAAAGAA   | TACCCGAGGA  | ACATGAGGTC  |
|    | 1951 | GTATATCAGCT | TCGAGAAGCT  | AGATCGGATG  | GTGCAAGTCC  | TCCCAGCCGA  |
| 60 | 2001 | AGTCCCTGCA  | GATTCAGCCA  | ATGATCTCTAC | GGGTCCGCTT  | CCTAATCCAG  |
|    | 2051 | ATAGTAACCC  | TGATTTCTTCG | CAAAATGAAG  | GCAGTTAG    |             |

The PSORT algorithm predicts inner membrane (0.2338).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 126A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 126B) and for FACS analysis.

- 5 These experiments show that cp6742 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 127

The following *C.pneumoniae* protein (PID 4376744) was expressed <SEQ ID 253; cp6744>:

- 10
- ```

1 VIQHLLNFAL EETPSISVQY QEQEKLSPCD HSPFEGKKKR WNKLEBSFSTY
51 CSLFMSVKDH YKLNLIQNS LSGWLLDPYR VCAPLSSPYE CPSYLLDLQN
101 KELRRSLLST FLDPKMLTSE TFRSVSINFG NSSFGQRWSE FLRSVLHDEK
151 EKHVAVVCND AKLLREGLSP EALSLEEDL RBSGYSYLNI LSVSPBGVSK
201 VQERQLLRD LQGRSFTVMI TDLPLGSEDI RSLQLASDRI LVSSSLDAAD
251 ACASGCKVLV YENPNAWQA ELENFYKQVE RRR*

```
- 15 The cp6744 nucleotide sequence <SEQ ID 254> is:
- ```

1 GTGATACAAC ATCTTCTAAA CTTTGCTCTA GAAGAGACCC CTTCATTTTC
51 CGTGCAATAC CAAGAACAAG AGAAGCTCTC TCCGTGCGAT CATTCGCCAG
101 AAATAGGTAA AAGAARAGA TGGAAATAGC TGAATCCTT CTCCACGTAT
151 TGTTCCTCTG TTATGTCTGT TAAGGATCAT TATAAGCTGA ATCTAGGAAT
201 TCAGAAATCC CTGTCAGGGT GGCTCTGGA TCCCTATAGG GTTTGCGCGC
251 CTTTATCTTC ACCGTACTCG TGTCCTTCTC ATCTTTTGA TTGCAAAAC
301 AAGAGAGCTAC GTGCTTCCCT TCTGCTAACG TTCTTAGACG CTAAAAATCT
351 CACTAGCGAA ACATTCGGT CTGCTCTAT AAACCTTGCG AACTCTTCGT
401 TTGGACAGAG ATGGTCAGAG TTTCTATCTC GTGTTCTGCA CGACGAGAAA
25 451 GAAAGACACG TAGCTGTTGT TTGTAATGAT GCAAACTTC TGGAGAAGG
501 AATTGCCCCA GAGGCAATGT CTCTATAGA AGAAGACTTA AGAGAATCAG
551 GGTATTCGTA TCTAAACATT CTCTCGGTGA GCCCCGAAG AGTCTCCAAG
601 GTTCAGGAAC GTCAATCTCT AAGCGAGAT CTCCAGGAC GGTCTCTTAC
651 TGTCAATGAT ACAGATCTCT CTTTAGGTAG CGAAGATATC CGTACTTTAC
30 701 AATTAGCCTC GGTAGGATT TTAGTCTCCA GTTCTCTTGA TGCCCGGAT
751 GCATGTCCTT CGGGAATGAA AGTCTTAGTC TACGAAAAAT CAAATGCATC
801 CTGGGCTCAG GAATTGAGA ACTCTACAA ACAAGTTGAG AGAAGAAGGT
851 AG

```

The PSORT algorithm predicts cytoplasm (0.3833).

- 35 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 127A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 127B) and for FACS analysis.

These experiments show that cp6744 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### 40 Example 128

The following *C.pneumoniae* protein (PID 4376745) was expressed <SEQ ID 255; cp6745>:

- 45
- ```

1 VACPSSISWP TVVRQHFWNA FDFTHFVCSR ITNPFALGIK AIPVLGHVIM
51 GLENLISWIP RHTVRHOMPT SVSSAIKVE QTRGHNCLAP LEAYLSLRLV
101 PISQEDLGKV HGRTPEDPFV DITPTEIVQL LPDELSTVD EALQVRSRL
151 TYAYRSVEKP MIQDLALVGF GLRDSADLIN FVRLANGVN HYPHTVKVLY
201 LAKNLADVWD CRISREKGGQ LRLGLDPRI ESISLTAGL PSVPFAVVD
251 PMITCGIDQ EVQDP*

```

The cp6745 nucleotide sequence <SEQ ID 256> is:

```

1  GTGGCTTGTC CRAAGTATTC TTCTTGGTTT ACTGTCGTTT GACAGCATTT
5  51  TGFNAACGCC TTGATTTTCA CCCATCCCGT TTGTTCTCGG ATTACAANTT
101 TTGCTTTGGG GATCATTAAG GCAATCCCGT TATTAGGACA GATTGTCATG
15  151  GGAATCGAGT GGTGATTTTC CTGGATTCOC AGACACAGCG TTGCTCATGG
201 AATGTTTACT TCTGATGTC CTAGTCTGAT TAAAGTAGAA CAAACACGGG
251 GTCATAATGG TTTAGCTCCC CTAGAAGCCT ATTTAAGTAG CTTGAGAGTC
301 OCCATTTCCC AAGAAGATCT AGGCAAAAGT CACGGGAGAA CCCCAGAGAT
351 TCCTCTCGTA GATATCACAC CCACAGAAAT TGTCCAACIT CTCCCTGATG
10  401 AAGAACTCTC TACTGTAGAT GAGGCACTGC AAGGCGTTTG TAGTAGGTTA
451 ACCTATGCGT ATAGGTCGGT AGAGAAACCT ATGATTCAAG ATCTTGCTCT
501 TGTGGGTTT GTCTCTCCAG ATCTCTCCGA CCTCATAAAT TTCGTGCGTC
551 TTGCTAATGG COTGCAGAA CACTATCCCC ATACTAAAGT GAAGCTCTAT
601 TTAGCGAAGA ACTTGCCAGA TGCTTGGGAC TGTGAAATTT CTGAAGAGGA
15  651 AALAGGCGAA CTCCGAGCTC TAGGTTTAGA CCCTAAATAA GAGAGTATAT
701 CCTTACGAG TGCAGGCTCT CCTTCACTGC CAGAAGTCGC TACTGTGAT
751 TTTATGATTA CCTGTTACGG AAGAATCAG GAAGTCCAAG ATCCCTAG

```

The PSORT algorithm predicts inner membrane (0.2253).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 128A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 128B) and for FACS analysis.

These experiments show that cp6745 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 129

The following *C.pneumoniae* protein (PID 4376747) was expressed <SEQ ID 257; cp6747>:

```

1  MMKQGVQDQA KELYTFLSRG NEHYQPCLMF SLEBELGLFL DEKMLCAPLS
5  51  EDHYCHSVLV DLVDQHLKDL ILSMFLDPQN ISAGELLKVS INVGDSPSFL
101 QOKDFLSMVL RDETGHNVVV VFGVLSLPA TVQCKLVEEL NSKDSYSLNI
15  151  PSCHGDSSPQ LLFRKELEGT SGREYPTVICA LYLGDITDMS LQLASERIMV
201 SREFDLVDAY AARCKLLKID INWNRPTFS RHADFAVDAD VSAGFNSREF
251 KLITQANQGI LESGELPLPS KTFWEGFLAF CDRVTVTRHF IPMLDAAIKQ
301 AVWTHKHPSL IDKECEALDL KTQCLPSIVS YLEVYVNSHE KTSKGPFIQK
351 EIIADCSPLK BALFPGSDED VPSTSEDPDS DHFSDLEDS*

```

The cp6747 nucleotide sequence <SEQ ID 258> is:

```

1  ATGATGAAAC AAGGAGTCGG GCAGGATGCT AAAGAGCTAT ACACATTTCT
5  51  ATCTCGTGGG AATGAGCATT ACCAACCGTG TCTATGGTCT AGTCTCGAAG
101 AGGAACTCGG ATTCCTTTTC GATGAAGAAA TGCTCTCGCG CCCTCTATCT
15  151  GAGGATCACT ATTGCCACTC GTATCTGTGA GATCTAGTGG ATCAACATTT
201 AAAGGATTTA ATATATATCGA TGTTTITAGA TCCTCAGAAAT ATCTCAGCAG
25  251  GAGAACTCCT CAGGTCCTCT ATAAACGTTG GAGATTCCTT TTCTCTCTFA
301 CAACAGAAAG ATTTCCCTCTC GATGCTCTTA CGTGATGAAA CGGGAAAGAA
35  351  CGTCGCTGTC GTTTTAAAG GAGTTCCTCT CTTACCCGCA ACCCAAGTCT
40  401  GCAAAATAGT AGAGGAATTG AACTCTAAGC ACTACTCCTA CCTCAATATA
45  451  TTTTCTTTGTC ACGGAGTAGT TAGTCTCTCA GATTTCTTTC GTTAAAGGAT
50  501  AGAGGGAAC TACGGGCGTT ATTTTACAGT GATTTGCGCT TTATATCTAG
55  551  GGGATACAGA CATGCGTAGT TTACAACTTG CTTCTCAAGG GATCATGGTC
601 TCTAGAGAGT TTGATCTTGT AGATGCCATCT GCTGCAAGAT GCAAGCTCTT
65  651  GAAAAATCAT CATACAAAAT GGAGACCTGG AACTTTCAGT CGCCACGCCG
701 ATTTCCGAGA TGCTGTAGAC GTATCAGCAG GATTTAACTC AAGAAGATTT
75  751  AAACATGATTA CGCAGGCGAA TCAAGGGATC CTAGAGTCTG GAGAACTCCC
80  801  GCTCCCTTCA AAAACCTTCT GGGAGGAGAT CTTAGCATTC TGTGATCGAG
85  851  TGACTGTAC GAGACACTTC ATTTCAATGT TAGACGCCGC TATAAGCAA
90  901  GCGGTATGGA CTCATAAACA TCCGAGCTTG ATAGATAAAG AGTGTGAAGC
95  951  CCTAGACTTG AAAACACAGT GCTTGCCATC TATGATATCG TACCTTGAAT
10  1001 ATGTCACAAA CTCTCAGGAA AAAACATCGA AAGGCGCGTT CATCAAAAAA
105 1051 GAGATTATCG CAGACTGTTC TCCTCTTAAA GAGGCGCTCT TCCACAGTTC

```

1101 TGATGAAGAT GTTCCCTCTA CCTCTGAGGA TCCTTCAGAT GATCATCCTT  
1151 CGGATCTTGA AGACICTTAA

The PSORT algorithm predicts inner membrane (0.1447).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 129A) and also as a his-tagged product. The recombinant proteins were used to immunize mice, whose sera were used in a Western blot (Figure 129B) and for FACS analysis.

These experiments show that cp6747 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 130

The following *C.pneumoniae* protein (PID 4376756) was expressed <SEQ ID 259; cp6756>:

1 MASGIGGSSG LGKIPPKDMG DRSRSPSPKG ELGSHEISLP PQEHGREGAS  
51 GSSHIHSSSS FLPEQESQS SSSAASPGF FSRVRSQVDR ALKSFNGNFFS  
101 ABSTSCARET ROAFPVRLSKT ITADERRDVD SSSAAATEAR VAEDASVSGE  
151 NPSQGVPEFS SGPEPQRLFS LPSVKQSGSL GRLVQTVDRD IVLPSGAPPT  
201 DSEPLSLYEL NLRLSLRLEQ LSDIQNDOL TPEBKAEATV TIQOLQITE  
251 FCQGYMEATQ SSVSLAEARF KGVETSDEIN SLCSLIDFE LQELMSDGS  
301 LQNLLEDAD LDEALSHTR LPSLSDNPT PIDNNPLIS QREPIYEEIG  
351 GAADPQRTRE NWSTRLNQI REALVSLGM ILSILGSLH RLRIARHAA  
401 EAVGRCCCTCR GEECTSSEED SMSVSGSPSEI DETERVGSFH DVPRRNGSPR  
451 EDSPLANLIV GWAHKGAKT KESSESSTPE ISISAPIVRG WSQDSVSFPI  
501 VMEDDHIFYD VPRKKGITVD VPSSPRMSPA RELEEDVFGD YEVPITSAEP  
551 SKDKNIYMTF RLATPAIYDL PSRPGSSGSS RSPSSDRVRS SSPNRRGVPL  
601 PVPVSPAMSE EGSYIEDMSG ASGAGESDYE DMSRSPSPRG LDEPIYANT  
651 PEDNPPTQRN IDRLQERSG GASAPVEPI YDEIPWCHGR PPATLPRPEN  
701 TITNVSRLVS PGPPEVRAA LLSESVSAVM VEAESIVPPT EPGDGRSEYL  
751 EPLGLLVATT KILLQKGWPR GESNA\*

The cp6756 nucleotide sequence <SEQ ID 260> is:

1 ATGGCATCAG GAATCGGAGG ATCTAGTGGA TTAGGAAGA TTCCACCTAA  
51 AGATAATGGG GATAGAAGTC GATCGCCCTC TCCTAAGGGA GAACCTGGCA  
101 GCCACGAGAT TTCCCTGCCT CCTCAAGAAC ATGGAGAGGA AGGAGCTTCA  
151 GGATCTTCGC ATATACATAG CAGTTCCTCT TTCTACACAG AAGATCAGGA  
201 GTCTCAGAGC TCTCTTCCGG CAGCTTCTAG CCGCGGATTT TTTTCTCCGG  
251 TACGTTCTGG GGTAGACAGG GCCTTAAAT CATTTGGCAA CTTTPTTTCC  
301 CGCAGCTCTA CGAGTCAAGC CGGTGAAAGC CGACAGACTT TTGTTAGATT  
351 ATCAAACACC ATCACCGCGG ATGAGAGACG GAGTTCGAT TCATCAAGTG  
401 CTGCTGCTAC AGAAGCCGGA GTGGCAGAGG ACCTGAGTGT TCTAGCGGAA  
451 AATCTTCTTC AGGGGGTTCG AGAAACCTCT TCTGGACAG AACCTCAGCG  
501 TTTATTCTCT CTCTCTTCTG TAAAAAACCA GAGCGGTTTG GGTCTGTTGG  
551 TACAGACAGT TCGCGATCGC ATAGTACTTC CTAGTGGGGC TCCACCTACA  
601 GACAGCGAGC CTTTAAGTCT CTACGAGCTA AACCTCGGTT TGAGTAGTPT  
651 ACGTCAGGAG CTCTCTGACA TACAAAGTAA TGATCAGTTG ACTCCAGAGG  
701 AAAAAGCAGA AGCCACAGTT ACCATAACAC AGCTGATCCA AATTACAGAA  
751 TTCCAATGCG GCTATATGGA GGCACACCAA TCTTGGTAT CTTAGCAGA  
801 AGCTCGTTTT AAGGGGGTAG AACTACAGTA TGAGATCAAT TCCCTCTGTT  
851 CAGAACTGAC AGATCTTGAG CTTCAGAAAC TCATGAGTGA TGGAGACTCT  
901 CTTCAAACCC TATTAGATGA GACTCGCCAG GATTTAGAAG CTGCTTTGTC  
951 CCAATACCTA TTGAGTTTTT CTTTAGACGA TAACTCAACT CGATAGACA  
1001 ATAATCCAC TCTGATTTCT CAAGAAGAGC CTATTATAGA GGAAATCGGA  
1051 GGAGCTGACG ATCCTCAAAG AACTCGGGAA AACTGGTCTA CAGGATTATG  
1101 GATCAGATT CGCGAGGCTC TGGTTTCTCT TTTAGGAATG ATTTTAAAGA  
1151 TTCTTAGGGCT CATCTTGACG AGSTTGCGTA TTGCTGCTCA TGCAGCTGCT  
1201 GAAGCAGTGG GTGCTTGTTG CACGTGCCGA GGAGAAGAT GTACTTCTTC  
1251 TGAAGAGGAC TCGATGTCCG TGGGGTCTCC TTAGAAATTT GATGAAACTG  
1301 AAGAAGCGGG CTCTCCGCAT GACGTTCCAC GCAGAAATTT AGTCCACAGT  
1351 GAAAGATTCT CATTTAGTAA TGCCCTTAGT GAGTGGGCAC ATAAGCACGG  
1401 TGCTAAACCC AAGGAGAGTT CAGATCARG TACCCCGGAA ATTTGATTTT  
1451 CTGCTCCCAT AGTGAGAGGT TGGAGTCAAG ACAGTTCGCT CAGTTTTATT



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1501 GTTATGGGAG ATGATCATAT TTTCATGATG GTTCTCGTA GAAAAGATGG  
 1551 AATCTATGAC GTTCCTAGTT CCCCTAGATG GAGTCTGCG CGAGAGTGG  
 1601 AAGAGGATGT TTTTGGAGAT TATGAAGTCT CTATAACCTC TGCCTGAACCA  
 1651 TCTAAAGACA AGACACTCTA CATGACACCT AGATTAGCAA CTCTCGTATC  
 1701 CTATGATCTT CCTTACCGTC CAGGATCGTC TGAAGCTCA CTTCTCCGCT  
 1751 CTTAGATGCG CGTACGAGC AGCTACCCAA ATAGACGGGG TGTCCCTCTT  
 1801 CCTCCAGTTC CTTCACCTGC TATGATGAGG GAGGGGAGCA TTTATGAGGA  
 1851 TATGAGCGGT GCTTCAGGTG CAGGTTGAAAG TGATTATGAA GATATGAGCC  
 1901 GTTCCCCCTC TCCTAGAGGC GACTTGGATG AACCCATATA TGCCTAATCT  
 1951 CCTGAAGATA ATCCATTATC TCAGAGAAAT ATAGATAGAA TTTTACAGGA  
 2001 GAGGTACGGC GGTGCTCCCG CTTCCTCGCT AGAGCTATT TATGATGAGA  
 2051 TCCCATGGAT TCAATGGCAGG CCCCCTGCTA CACTCCCAAG ACCCGAGRAT  
 2101 ACATTGACTA ATGTTTTCGT TAGAGTGAGC CCAGGGTTTG GACCAGAAAT  
 2151 AAGAGCCGCT TTGCTTAGCG AGAGCGTGAG TGCTGTTATG GTCCGAAGCAG  
 2201 AGAGTATTCT TCCTCCCAACA GAGCCGGGGC ACGGAGRAT AGAATATCTA  
 2251 GAGCCCTTAG GGGGACTTGT AGCTACAAG AAAATCTTAC TACAAAAGG  
 2301 ATGGCCTCGT GGAAGATCGA ATGCTTAG

The PSORT algorithm predicts inner membrane (0.3994).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 130A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 130B) and for FACS analysis.

These experiments show that cp6756 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 131

The following *C. pneumoniae* (PID 4376761) was expressed <SEQ ID 261; cp6761>:

1 MTVAEVKGTFLVCLGCRVNYQEVQAYRDLTLILGYQEVLDSEIPADLCI  
 51 INTCAVTAESAESSRHVRVLCRQNPATIAIVVTGCLGESDKEFPASLDQR  
 101 CTLVSNKEKSLRIEKLFSYDTTFPEFKIHSFBGKSRAPFKVQDGCNSFCS  
 151 YCIIPLYRGRSVSRPAEKILAEIAGVVDQGYREVVIAGINVGDCDGRS  
 201 LASLIEQVDRIPGIERIRISIDPDDITEDLHRAITSSRH TCPSSHLVLQ  
 251 SGNLSILKRMNRKYSRGDFLDCVEKFRASDPYAFPTDVI VGFPGESDQD  
 301 FEDTLKILIEDVGFILVHSFESARRRTKAYTFDNQIPNQV IYERKKYLAE  
 351 VAKRVGQKRM MKRLGETTVEV LVEKVTGQVA TGHSPYFEKV SFPVVGTVAI  
 401 NTLVSVRLDRVEEGLIGET V\*

The cp6761 nucleotide sequence <SEQ ID 262> is:

1 ATGACGGTTGCGGAAGTCAAAGGAACATTAAAGCTGGTCTGTTTAGGCTG  
 51 TCGGGTGAATCAGTATGAGGTCCCAAGCATA TCCGACCCAG TTGACTATCT  
 101 TAGGTTACCAAGAGTCTCTGATTCTGAAA TCCCTGCAGA TTTATGATCA  
 151 ATCAATACGPTGTCTGTCTACAGCTTCVGTCTGAGAGTTCGG GTCTCATGCT  
 201 TGTGCTCAGTTATGTCTGTAGAAACCTATCAGCATATATTGTGTACAGC  
 251 GTTGTATTGGGGAATCTGACAAAGAGTTT TTTGCTCTTT GGATCGGCAA  
 301 TGCACACTTGTTTCCCAATAAGAAAATCCGCACTATATAG AAAAATTTT  
 351 TTCTTATGATACGACCTTCCCTGAGTTCAA GATCCATAGT TTTGAGGGAA  
 401 AGTCTOGAGCTTTTATTAAAGTTCAAGATGCTGTAAATCT TTTTGTCTCG  
 451 TACVGCATTA TCCCTTATTTT GCGGGGGCGT TCGGTTTCTC GTCTGTCTGA  
 501 GAAGATTTTA GCTGAAATCG CAGGGGTTGT AGACCAAGGA TATCGCGAAG  
 551 TTGTAAATGGAGAAATTAAT GTTGGAGATT ATTGCGATGG AGAGCGTTCA  
 601 TTAGCCTCTT TGATTGAACA GGTGGACCGG ATTCCTGGAA TTGAGGAGAT  
 651 TCGAATTTCCCTCTATAGATCTGATGATAT CACTGAAGAT CTGACCGGTG  
 701 CCATCACCTC ATCGCGTCAACCTTGTCTCTT CGTCAACCT TGTCTCTCAA  
 751 TCGGGGTGGAATTCATTTT AAAGAGATG AACCGGAAT ATTCTCGCGG  
 801 AGATTTTTGA GATGTGTAG AGAAGTTCG TGCTTCTGAT CCTCGCTATG  
 851 CCTTTACTAC AGATGTGATT GTCCGATTTCTGAGAGAG TGATCAAGAT  
 901 TTTGAAGATA CTTTGAAGAT TATTGAAGAT GTAGGCTTATA TTAAGTGCA  
 951 TAGTTTCCCT TCTAGCTGCT GTCGTGTGAT TAAGAGCAT ACTTTGATA  
 1001 ATCAGATTCCCAATCAGGTG ATCTATGAGA GGAAGAAGTA TCTTGTCTGAG  
 1051 GTTCTTAAAGGGTAGGCCA GAAAGAGATG ATGAAGCGTT TAGGAGAGAC

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1101 TACAGAGGTG CTTGTTGAGA AAGTAACGGG CAGAGTTGCT ACGGGTCACT
1151 CTCCTTAATT TGAAGAAGGT TCTTCCCTG TTGTAGGAAC GGTAGCTATC
1201 AACACTCTAG TTTCTGTGCG TCTTGATAGG GTAGAGGAAG AAGGGCTGAT
1251 TGGGAGATTT GTATGA

```

- 5 The PSORT algorithm predicts inner membrane (0.1574).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 131A) and also as a his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 131B) and for FACS analysis.

- These experiments show that cp6761 is a surface-exposed and immunoaccessible protein, and that it  
10 is a useful immunogen. These properties are not evident from the sequence alone.

### Example 132

The following *C.pneumoniae* protein (PID 4376766) was expressed <SEQ ID 263; cp6766>:

```

1 MATSVPTVSS TSVGRANSSN ERFTERTSRM YYAALVLGAL SCLIFIAMIV
51 IFFPUGLNAV VLFGLFGCLL LSLAIVFAVS GLVGLKTLER SREATPPEIV
101 AQKEWTTQDD VLGNEYWRSE LLSLFLRGDL HESLIVDSKD RSLDIDQSLQ
151 NILKLEPLST TSLSLKKDCV HINIILHLVR QMWLLGVDLR PEVTAHADEL
201 LLLFIEEQYV SPDLKLLIRY GDALQATSPL MDWADSGSFS VDADGVFSCR
251 REECSPEDAL AQFDLLLEAL NPDRRLKDS FLVYIWSSSF FEKFLHRHLE
301 SLQKRLPETA IDVARYEAQI QTFLSRYFOK LDLINAMSLD WGNYNABEGEK
401 ILNENSGFLC SLVEYPLSYL IDMAVLDDCV RGTETSLDEQ ADYTVCLQGL
451 DSNLSQSFAS LQSGQKVLNP RDVLSEQAAR MLVHGLAAQG VSFQGLKALM
501 YLTAVPQRMW LGALPLFESF FVFNRMKEFL GESLGD*

```

The cp6766 nucleotide sequence <SEQ ID 264> is:

```

25 1 ATGGCAACCT CTGTTCCGTGT AACTCTCATCT ACTTCGTAG GAGAGGCTAA
51 CTCCTCCCAAC GAAAGATTTA CTGAACGAAC ATCCGGAATG TATTACGCAG
101 CTTTACTGCTT AGGGGCTTTG AGCTGTTTAA TTTTATTTGC TATGATTGTC
151 ATTATTCCACAC AGCTCGGATTG GTGGGCTGTG GTCCCTCGGT TTGCTCTTGG
201 ATGTGTTACTT TTAAGCTTAG CTATCGTTTI TGCTGCTCCG GGTCTGTTT
301 TAGGCAAGAC TTTAGAACCT AOTCGAGAGC CGACTCTCC AGAAATTTGT
351 GCGCAAAAGG AGTGGACTAC ACAACMAGAT GTCTTAGGGA ATGATATTGT
401 TGATTGTTGA TCTTAAGGAT CGATCTTTAG ATATTGATCA GAGTTTACAA
451 AATATATTGA AACTTGAGCC CCTATTACG ACATCTTCG TGTTAAAGAA
501 AGATTCTGTC CACATCAATA TCATTTTACA TTTAGTGAACA CAGTGGAACT
551 TACTGGAGT GGATCTTAGT CCTGAAGTCA CTGCGCACGC CGAGGAATCT
601 CTACTCTTTT TGATAGAAGA GCGATPATTAC TCTCTGTATA TTTTGAATTT
651 GATTCTGCTAC GGAGATGCTT TACAGCAACG GTCTCTCTTG ATGGAATGGG
701 CAGATTTCAGG TCTCTTTAGT GTAGACGCGC ACGGGGTATT TAGCTTTCGC
40 751 AGAGAAAGAT GTTCTCTCTGA GGATGCTTTG CGGCAATTCG ATCTCTTTTT
801 GCGCTTTGGA AATCCGACCA GACGCTTCTT AAAGGATTCT TTCTTACCT
851 ACATTTTGGT GTCCTCATAT TTTTGAGAAT TTTTACATCG CCATCTAGAG
901 AGCTTGCAAA GAAAGCTCC AGAGAAGCGC ATCGATGTCG CCGCTATAGA
951 AGCAACAATA CAACAATTTC TCTCTCGCTA TTTTTCAGAG CTCGATTTGA
45 1001 TAAACCCAAAT GTCTTAGAT TGGGGATATA ACTGTGCTGA GGGAGAAAAA
1051 TGTATTGAGA GCGCAATCA AAGATTAGAC AACCTATTTA TTGCTTTTTC
1101 TCTCTCTGTT CTGCTATAGA AGCGGCTCTT TGAACAATAT GGTCTGTGG
1151 TACGGGTAGA TCGTAGGCAG ATCTCGTAGC AGATTCTTTC GAACACTGAA
1201 ATCTTAGAAA ATGAGTCAAG GTTCTCTGCG AGTTTGTATG AATATCCTTT
50 1251 ATCTTATTGT ATGAGATTGG GTGTTTGTCT AGACTGTGTT CGCGGTACCG
1301 AAATCTCTCT AGAAGATCAG CCGGATTACA CCGTTGTGTT GCAAGGCTTG
1351 GATTCTTATG TATCTCAATT TCGGAGTCGT TTACAGTCTG GACAAAAAGT
1401 ATTGAATCTT AGAGATGTTT TAAGTGAACA GGCTGCGGTT ATGCTTGTTC
1451 ATGGCTTTGGC AGCAGAGGCG GTGTCGTTTC AAGGATTGAA AGCTTTGATG
55 1501 TATTTGACAG CCGTTCCCCA AAGAATGTGT TTAGGAGCAT TGCTTTTATT
1551 TGAATCTTTT CCTGTCTTTA ATCGGATGAA AAGATTTCTT GGGGAATCTC
1601 TGGGAGACTA G

```

The PSORT algorithm predicts inner membrane (0.6158).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 132A) and also as a his-tagged product. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 132B) and for FACS analysis.

- 5 These experiments show that cp6766 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 133

The following *C.pneumoniae* protein (PID 4376804) was expressed <SEQ ID 265; cp6804>:

10  
 1 MSNQLQPCIS LGCVSYINSF PLSLQLIKRN DIRCVLAPPA DLLNLLIEGK  
 51 LDVALTSSLG AISHNLGYVP GFGIAANQRI LSVNLYAAPT FNFSPQPRIA  
 101 ATLESRSSIG LLKVLGRHLM RIFPHILRF ITTKVLKQTP ENYDGLLIG  
 151 DAALQHPVLP GFVYDYLASG WYDLTKLPFV FALLHSTSW KEHPLNLAM  
 201 EEALQGFESS PEEVLKEAHQ HTGLPPLSLQ EYVALCQYRL GEEHYESPEK  
 251 FREVYGTLYQ QARL

- 15 The cp6804 nucleotide sequence <SEQ ID 266> is:

1 ATGTCCTAAC AACTCCAGCC ATGTTAAGC TTAGGCTGCG TAAGTTATAT  
 51 TAAATCCTTT CCGCTGTCCG TACAACCTAT AAAAGAAAC GATATTGCGT  
 101 GTGTTCTTGC TCCCCCTGCA GACCTCCTCA ACTTGCTPAAT CGAAGGGAAA  
 151 CTCGATGTGT CTTTGACCTC ATCCCTAGGA GCTATCTCTC ATAACCTGGG  
 201 GTATGTCCTCC GGCTTTGSA TGCAGCAA CCAACGTATC CTCAGTGTAA  
 251 ACCTCTATGC AGCTCCCACT TTCTTTAACT CACCGCAAC TCGGATTGCC  
 301 GCAACTTTAG AAGTCCGCTC CTCTATAGGA CTCTTAAAG TCGTTGTGCG  
 351 TCATCTCTGG CGCATCCCAA CTCCTCATAT COTAGATTTC ATAACACAA  
 401 AAGTACTCAG ACAACCCCT GAAATTTATG ATGGCCTCCT CCTAATCGGA  
 451 GATGCGAGCG TACAACATCC TGTAATCTCT GGATTGTGTA CCTATGACCT  
 501 TGCTTCGGGG TGGTATGATC TTACAAAGCT ACCTTTTGTA TTTGCTCTTC  
 551 TTCTACACAG CACCTCTTGG AAAGAACATC CCTTACCCAA CCTTGGGATG  
 601 GAAGAAGCCC TCCACAGTGT CGAATCTTCA CCCGAAGAG TCCTTAAAGA  
 651 AGCTCATCAA CATAACAGTC TGCCCCCTTC TCTTCTTCAA GAATACTATG  
 701 CCTATGCCCA GTACCGTCTA GGAGGAAGAC ACTACGAAG CTTTGAAAAA  
 751 TTCGGGGAAT ATTATGGAAC COTCTACCAA CAAGCCGAC TGTA

The PSORT algorithm predicts inner membrane (0.060).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 133A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 133B) and for FACS analysis.

These experiments show that cp6804 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 134

The following *C.pneumoniae* protein (PID 4376805) was expressed <SEQ ID 267; cp6805>:

40  
 1 MSSLLSCGRI EPTRVTCSLK TYLEDTSONQ LSTRLVASV IFLCALLIIL  
 51 VCVALSSSLIP SIMALATSFT VMGLILFVMS LLGDVALISY LTYSTVTSYR  
 101 CNKRAFETHK PARSVYEGV RHWDLGRSLI GTGEIPIVRT LFSPPQNHGL  
 151 NHALAAKIFL FMEHFSPEPP NEPLVDNACL IRDFRPHVSS LCFVIEKQGS  
 201 SLRKTGNTI CBAFRSDYDA HFAMVDCYRL IHSKLIIEKM GLKNIDIIPS  
 45 251 VMVREDYPSR PGGYREGLL RMYGKGAL\*

The cp6805 nucleotide sequence <SEQ ID 268> is:

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1 ATGTTCATCAC TACTGAGCTG CGGAAGAATA GAGCCGACTC GGGTTACCTG  
 51 TAGCTTAAAG ACGTATCTTG AGGATACGAG TCGAATTCAG TTGAGCACAC  
 101 GTCAGTTCGG GCGAAGTGTG ATCTTTTATAT CGCGATTTGT GATCATTTTG  
 151 GTTGTGTGGG CCCCTCTAG TTTGATTCGA AGCATTTAGG CCTTGGGAGC  
 5 201 CTCCTTTACG GTAAATGGGGT TAATCTTTT TGTGATGTCA CTCTCTGGTG  
 251 ACGTTGCAAT TATAAGTTAT CTTACTTATA GACATTTGAC GAGTACCGG  
 301 CAAATTAAGA GAGCTTTTGA GATTCACAG CCCGCTCGCT CCGTTACTTA  
 351 CGAGGGGGTC CGCCATTGG APTTAGGACG ATCATCTTGA GGCACAGCGG  
 401 AGATTCCATAT AGTAAGGACG TTTATCTCTC CACTTCAGAA CCTGGTCTCT  
 10 451 AACCATGCTC TAGCTGCTAA AATTPTCTTA TTTATGGAGC ATTTACGCC  
 501 TGAGCCACCG AACGAGCCTT TGGTGGATTG GGCTGTGTGT ATTCCGGATT  
 551 TTAGGCGCTCA CGTCACTTCT TGTGCTTTG TTTATGA AAA ACNAGGGTCA  
 601 TCCCTGAGGA CTAAGGAAGG CAATACGATT TGTGAGGCTT TCCGCTCTGA  
 651 TTACGACGCC CATTTTGCTA TGGTAGATTG CTACCGGTG ATCCACTCTA  
 15 701 AGTTGATTAT AGAGAAATG GGAATGAAGA ATATCGAATT CATTCGAGT  
 751 GTCATGGTTC GTGAAGATTA TCCTAGCCGT CTGGGGAGG GGTATCGCGA  
 801 AGGCCATTAT CGTATGTATG GTGGCAAGG GGCTCTGTGA

The PSORT algorithm predicts inner membrane (0.711).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 134A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 134B) and for FACS analysis.

These experiments show that cp6805 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 135

25 The following *C. pneumoniae* protein (PID 4376813) was expressed <SEQ ID 269; cp6813>:

1 MSGPSRTESS QVSVLSVVR DKELAPKKQF TIAKISTLAI LASLALGALV  
 51 AGISLTIVLG NFVFLALLIT TALESVVTFL VYHMTSKVS SNWQKVLQEN  
 101 FKPLGKAWQE KNVDCYSNEM QFVNNHLPK FKVAIQTDAS QPFQPTFLVIG  
 151 LRVLKQNST GLIFNPVPGT NLINDATNLN STLLYSTLKD KSVWDTCKQR  
 201 BGGPAKGBDP PSPTEVRVVK LPNEALDQTF NLNLSAEEK SILPTFLGHV  
 251 CGPKSEELPN QQEVYRQALL AYENCLAAAI BSHRAIVLPL LPTSVYRVEPP  
 301 BEILPKEGTF YMDNQTAFC KRALLDAIQN TALRYPQRSL LVILQDPFNP  
 351 IESQSRSEB\*

The cp6813 nucleotide sequence <SEQ ID 270> is:

35 1 ATGTACAGGAC CCTCACGTAC TGAGAGCTCT CAAGTTTCTG TACTATCCTA  
 51 TGTGCCCTCGG GATAAAGAAA TTGCTCCTAA AAAACAGTTT ACCATAGCRA  
 101 AAATAATCCAC TCTTGCAATC CTAGCTTCTT TAGCPTTAGG AGCTTTGGTG  
 151 GCTGGAATCT CTTTAACGAT AGTATTAGGG AACCTGTAT TTTTGGCTCT  
 201 CTCTCATTACC ACGGCCCTCT TCTCACTTGT AACCTCTTGA GTCTACCAAC  
 40 251 AAATGACCTC AAGGCTATCT TCTAATGCTG AGAAAGTTCT AGAGCAAAAC  
 301 TTCAAGCCTT TGGGAANAAG GTGGCAAGAA AAAAAGCTAG ACTGCTACTC  
 351 AAACGAGATG CAATTTTACA ATATCACTCT GAACCTTAAG TTCAAGGTAG  
 401 CGATACAAAC AGATGCGTCT CAACCATTTT AGCTCACTTT CTTAATGGGA  
 451 CTTAGAGTGA TCGAAAAAAA TCAATCCACA GGGATCACTT TTAATCCCGT  
 501 AGGCCCAACG AATCTGATCG ACAACACTGC AACGACCTCT TCTATATCC  
 551 TTTACTCCAC CCTAAAAGAT AAAAGCGTGT GGGATACATG CAAGCAGCGC  
 601 GAAGGGGGTG CCGCAAAAAG AGAAGACCCC TTTTCCCTTA CCGAAGTGAG  
 651 AGTAGTAAAA CTTCCAAACG AGCTCTAGA TCAAAAGTTT AATCTAAAT  
 701 TAAGCTCTGC AGAAAAAGAA AGTATTTCTT CGACCTTTT AGGCCACGTA  
 50 751 TGGCGCCCTA AATCTGAAGA GTTACCAAT CAGCATGAT ATTATCGCCA  
 801 AGCTTTACTA GCGTACGAGA ACTGCTTAA AGCAGCTATP GAAAGTCATG  
 851 CAGCAATCGT TGCTCTTCTT CTCTTTACTT CGGTCTATGA AGTGCTCCGA  
 901 GAAGAGATTC TTCTTAAAGA AGGCACCTTC TATTGGGACA ACCAACTCA  
 951 AGCGTTTTCG AAACGCGCTT TATTGGACGC TATTCAAAAT ACGGCCCTAC  
 55 1001 GCTATCCTCA AAGATCTTTA CTGTATTATC TCCAAGATCC TTTTAACTACT  
 1051 ATAGAATCAC AAGATCGTTC TGAGGAGTAA

The PSORT algorithm predicts inner membrane (0.4291).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 135A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 135B) and for FACS analysis.

- 5 These experiments show that cp6813 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 136

The following *C.pneumoniae* protein (PID 4376844) was expressed <SEQ ID 271; cp6844>:

```

10      1  MWRVVLRLFI  IFILGRAVFF  LRASEFSWE  TSTCLTVLGI  PFIDILITN
      51  EDFVAQCGLQ  IGTISSTNNA  KIKEIFLIYK  EKFPASISF  KRKEFLNLSQ
    101  SHLSDLGLIC  MRNGEYVAG  MANKENGPA  KQPKDLRLVL  RCPNPDTLL
    151  YSEKEAEKGI  ETNTCLCNQ  YTLIDGLIL  YGDSIEKFLK  ETRKNNNHTL
    201  VDLCDQSVVT  TFLGRFWSLL  NYVQVFLSE  DSAKILAGIF  DLAQATQLLS
    251  HTVPLLIYIT  NDSIHILEQ  KESSFTYNQ  D  LTEPILGFLF  GYINRGSMY
    301  CFNCAQSLG  ET*
```

The cp6844 nucleotide sequence <SEQ ID 272> is:

```

20      1  ATGTGGCGCG  TTGTCCCTAG  ATTCCTTATA  ATTTTATCT  TGGGAAGAGC
      51  CGCTTCCCT  CTAAGAGCTT  CAGAAAGCTT  CTCCTGGGAA  ACATCGACCT
    101  GTTTAACAGT  GCTAGGATTT  CCTTTCATAG  ATATTATCCT  CACAACGAAT
    151  GAGGACTTTG  TTGCCAGTGT  CGGCTCGCAA  ATAGGAACCA  TTCTCTCGAC
    201  TAATAACGCA  AAAATAAAG  AAATTTTTTT  GATATATAG  GAAAAATTTC
    251  CAGAAGCCTC  TATCAGTTTC  AAACGAAAG  AACCTCTAA  CCPTTCCCAA
    301  TCCCATCTCT  CCGATTTAG  TATTTTATGT  ATGCGTAA  GAGAAACTTA
    351  CGCTGAGGGA  ATGGCAAATA  AAGAAAAAG  ACCCGCTCTA  AAACAACCCA
    401  AGGATCTAAG  ATAGTTTTA  CGTTGTCTTA  ACCAACGAGA  TACCCTGCTC
    451  TACTCGGAAA  AAGAAGCAGA  AAGGGCATA  GAAACAAATA  CTTCCTATG
    501  CAATCAGGGA  TACACATCC  TGGATGGGCA  ATTGATTCTC  TACGGGATA
    551  GTATAGAAAA  GTTCTGAAA  GAGACAAAA  GAAAGATAA  CCACACGCTT
    601  GTTGATCTTT  GTGACTCACA  AGTCGTGACC  ACGTTCCTCG  GTCCGTTTTG
    651  GTCTCTCTTA  AACTACGTT  AAGTTCTTTT  CCTATCTGAA  GACTCCGCTA
    701  AAATCTTTGC  GGGCATCCCA  GACCTAGCTC  AAGCTACGCA  ATTGCTTTCC
    751  CACACCGTAC  CTTTGCCTTT  TATTATACCA  AACGATTCTA  TTCACATCAT
    801  AGAACACAGG  AAGAAAGTA  GTTTTACCTA  TAACCAAGAT  TTAACAGRAC
    851  CCATTTTAGG  ATTTCTCTTT  GTTTACATAA  ATCGCGGCTC  TATGGAATAC
    901  TGCCTTAATT  GTGCACAGTC  TTCATTAGGA  GAAACCTTAA
```

The PSORT algorithm predicts inner membrane (0.1786).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 136A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 136B) and for FACS analysis.

- 40 These experiments show that cp6844 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 137

The following *C.pneumoniae* protein (PID 4377201) was expressed <SEQ ID 273; cp7201>:

```

45      1  VLVGICPSLY  PEHRSFYFR  VSGDIGSRFD  DRGFVNSGVE  TLPYSSGSFG
      51  IFWISPTDPT  NFNAIVNFM  RTAGINEVSR  PMTQDTETSL  IEMRDLSEQQ
    101  EANNDSLEQ  EESLMGIYGH  TVGVSVMVT  SSPNIFYRIQ  TLLGLPETLA
    151  EARENPTFPN  STIDSLAEIN  MNLVRISDAV  SIFWIFPIVD  TTYNGVLLAV
```

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201 CIGFFGINGI CSTFLMLTNP RSRRDRNRNL RIMVLCYRSL GSGNLFDSL  
 251 NNVRMAARRH VTSCVALYA MVLFGMTVA IQDALQYGF SVRDIFYRYC  
 301 LRHRYCLTQR NEDSLQTTOF RQVQIRCHLE DQKMVASILN LSVPLGFPFGF  
 351 VGLMTTFGGL EISFSCRWDA ANNRVTGIF\*

5 The cp7201 nucleotide sequence <SEQ ID 274> is:

1 GTGCTCGTTG GTATCTGTCC TTCTCTATAT CCAGAACATC CTCGCTCCTT  
 51 TTAATTATCGT GTTCTTGGAG ATATAGGCTC CCGATTGCAG GATAGAGGAT  
 101 TTGTAAACCT TGGAGTCGAA ACCCTGCCAT ACTCTTCAGG CAGCTTTGGG  
 151 ATTTTITGGA TCTCTGTATC GGATCCCACT TTAAATTTTG CTATCGTAAA  
 201 TACCTTTATG CGAACTCGAG GGATCAATGA AGTCTCTAGA CCGATGACAC  
 251 AAGATACAGA AACTTCATTG ATAGAAATGA GAGACCTAAG TGAACACAA  
 301 GAAGCGAATA ACACAGATTC TTTAGAGCAA GAAGAGAGCT TAATGGGTAT  
 351 TGTAGGACAT ACTGTGGGAG GAGTTTCCAT GACCGTGACC TCAGTCCCAA  
 401 ATATCTTTTA TCGTATACAA ACACCTCTGG GACTGCCAGA GACTCTTGCA  
 451 GAAGCTGAAG AAAATCCTAC CTTCGCCAAT TCTACTATAG ATAGCCTTGC  
 501 AGAAATTAATG ATGAACCTCG TAAGGATCTC TGAGCTGTGC TCTATTTTCT  
 551 GGAATTTTCC TATCTAGAT ACTACATATA ATGAGATTTT ATTAGCCGTC  
 601 TGTATCGGCT TCTTCGGAAT CAATGGGATT TGTTCACGCT TCCTTATGCT  
 651 TACGAATCCA CGCTCTCGTC GAGATAGATG GAGGAATTTA CGCATCATGG  
 701 TTCTTTGCTA TCGTCTTTTG GGAAGCGGAA TGAATCTCTT TGATCTTAAG  
 751 AATAATGTGC GCATGGCAGC ACCTGAGGAT GTGACATCAT GTACAGTAGC  
 801 TCTCTATGCT ATGCTCACTC TATTTGATG GACAGTAGCA ATACAAGATG  
 851 CTTTGCAATA TGGTTCCCTT AGCGTTCCGG ATGCCTTCTA TAGATATTGC  
 901 TTACGCGACA GATATTGCTT AACTCAAGA AACGAAGACT CTCTGACAC  
 951 TACAGGAACG CGCTTTTCAG TACCCGATC ACATCTAGAA GATCAACAGA  
 1001 TGGTGTGCTT TATTTTGAAT TTGAGTCTTT TTGGGCTCTT TTTTGATTCT  
 1051 GTAGGGCTAA TGACACGTT TGGAGGATTA GAAATCTCAC CATCTGTGTC  
 1101 TGGGATGCA GCAATAACG GAACGGTAGG TATTTTTAG

The PSORT algorithm predicts inner membrane (0.3102).

30 The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 137A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 137B) and for FACS analysis.

These experiments show that cp7201 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### 35 Example 138

The following *C.pneumoniae* protein (PID 4377251) was expressed <SEQ ID 275; cp7251>:

1 MAPIHGNSAF VEDILHSHPS FQATYFSSTR AQLLHEFKDR HPVLTIRIADV  
 51 IIRKPKVLIG LIILPLGIYW LQCTCTWNSI LPSKNLLKIF KKPQWTKTLK  
 101 TNYLHALQDY SSKNRVASMR RVPILQNVLI IDTLEICLSQ APTNRWMLIS  
 151 LSGSDSLEBI ACKEIFDSWQ RPAKLIGANI LVYNNPGVMS STGSSSLKDL  
 201 ASAHNICTRY LKDKRGPGGA KRIITYGYSL GGLIQEALRL DQKIVANDPT  
 251 TWIAVKDRCP LFISPRGFHS CRRIGKLVAR LPWGVTAVE RSQDLPCLEI  
 301 FLYPFDLSRR STVRONKLLA FELTLAHAK NSPVVQNKHF IEVRLSSDID  
 351 PIDSKTRVAL ATPILKKLS\*

45 The cp7251 nucleotide sequence <SEQ ID 276> is:

1 ATGGCTCCAA TTCACGAAG TAAATGCGTTT GTTGAAGATA TTTTACATTCT  
 51 CCACCCCTTCT CCACAGCGA CTTATTTTTC TTACACACGC GCCCAAAAC  
 101 TTATCATGATT TAAAGACAGG CATCCCGTGC TTACACGAGT TGCTCTGTGA  
 151 ATTATTAATA TTTTAAAGT TCTGTATGGG CTGATCATCC TTCCCTTAGG  
 201 AACTCTACTGG CTATGTCAAA CGCTTTGACT AAACATCGATT CTCCTTCCCA  
 251 AGAATTTATT AAAAATTTTC AAGAGACAC CCACACATAA AACCTTAAAA  
 301 ACTAATTAAT TGCTATGCTT GCAAGATATT TCTCGAAAA ACCCGGTTCG  
 351 TTCCATGAGA CGAGTTCCTA TCCCTCAGGA TAAGTCTTCT ATCGACACTT  
 401 TGGAAATATG CCTTTACAA GCACATACGA ATCGTTGAGT GCTCATTTC  
 451 TTAGGAAGTG ACTGTAGCTT GGAAGAAATC GCTGTAAAG AGATCTTTGA

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501 TTCTTGCGCA AGATTTGCCA AGTTGATAGG GGCCAATATA CTCGTTTATA  
 551 ACTACCCCGG AGTCATOTCC AGCACAGGGA GCAGCAGCCT AAAGGACCTA  
 601 GCATCAGCTC ATAATATTGG TACAAGATAC CTTAAGAGATA AAGAACAGGG  
 651 CCTTGGAGCA AAGAATATCA TTACCTATGG GTACTCCCTA GGAAGTTTGA  
 701 TACAAGCAGA AGCAATTGCGA GACCAGAAGA TTGTTGCAAA CGATGATACT  
 751 ACTTGGATAG CAGTCAAGA TAGGTGTCCCT CTCCTTATAT CTCACGAAGG  
 801 TTTCACACGT TGCAGACGCA TAGGAAAGCT AGTAGCTCGT CTTTGTGGCT  
 851 GGGGAGCCAA AGCCGTAGAG AGAAGCCAAG ACCTTCCTCG CTAGAATAT  
 901 TTCTCTATAT CTACCGGATCT CTTACGAAGA TCAACAGTCA GACAGAACAA  
 951 GCTCTTAGCA CCTGAACTTA CTCCTGCTCA TCGGATAAAA AATAGTCCCT  
 1001 ATGTTCAAAA TAAAGAATTT ATAGAAGTAC GATTATCGTC TGATATCGAT  
 1051 CCCATCGACA GCAAAACAAG AGTGGCTCTT GCCACACCAA TTTTGAZAAA  
 1101 GCTCTCTTAG

The PSORT algorithm predicts inner membrane (0.4545).

- 15 The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 138A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 138B) and for FACS analysis.

These experiments show that cp7251 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

## 20 Example 139

The following *C. pneumoniae* protein (PID 4377288) was expressed <SEQ ID 277; cp7288>:

25 1 MHMSNPISLF SPAELIAKYN LIPKTSPIYP RRELTILEE NACQTRLNV  
 51 AQVLHPSSLF SMSKKILNFC GCSGGPLCWV ILNLIAFIIT SVLFILLPV  
 101 NLIVAGLRFL MPLPPKIVE DLSEPTTEET NEVIQPFIFA LQALLFEDNK  
 151 LRSEFKIVEQS VQKALPNPF LNRLVAISQP ESQEMRKIP DLCSQLKXVL  
 201 KSLGVLTFEW KHLKLYFEG LKNEHDSNPK KTFPILIKLL IEALTKXSL  
 251 PKTPSTKERM QALFIASSC KTCRPTWGEV ITRSLNRLYS IANEGUNQLL  
 301 IWWQEFKERE LMSIQDGDGA BEYRFAAQGH GERYTEALIE VLRNESAALK  
 351 QWHVINTMKF PHGNLGLVT EHLQDTLGLAL TLRYQTVDTH QGRSDADLSA  
 401 ALPLNKLYNS GNQLVNSVFK SMQRADPETK ALIREPALDI LYASLRPLQT  
 451 SAHTEVFSTL LMDPETVEPN KACIAYLLV LKILEL\*

The cp7288 nucleotide sequence <SEQ ID 278> is:

35 1 ATGCATATGT CTAACCCCAT CTCCTTGTTT TCCCCTGCAG AGTTAATAGC  
 51 AAGATACAAT TTAATTCCAA ARACTTCGCC GATTATATCT CGGAGGACGG  
 101 AACTTATTAT CTGGAAGAA AATGCGGTGC AAACACGCCT AACCACGTG  
 151 GCTCAGGTCC TACATCTCTC TAGCCTATTCT AGTATGTCAA AAAAAATACT  
 201 GAATCCCTCG GGGTGCTCTG GTGGTCCCTT ATGTGGGTG ATTCCTCAAC  
 251 TCCTAGCAAT TATTATTACT TCAGTACTGT TTATCATTCT TTTACCGGTG  
 301 AATCTCATCG TAGCAGGTCT TCGTCTCTTC ATGCCCTPTC CCCCTAAAA  
 351 AATCOTAGAG GATTTAAGTG AACCTACTAC TGAAGAACAG AATGAGGTCA  
 401 TTCACCCCTT CATTTTCGCT TTGCAAGCGT TGCTTTTGA GGAATAACAA  
 451 CTTGCGCTCT TTAATAATGT TGAACAAAGT GTAGGCAAG CACCCTTACC  
 501 TAATCCCTTT TTAATAAGAC TAGTAGCAAT TTCCGCCGAA GAAAGCCAG  
 551 AAGCCATGCG GAAGATTCCG GATCTATGCT CACAACTGAA AAAAGTATTA  
 601 AAGTCTCTAG CGGTGCTAAC TCCGAATGG AAGCAATGG TGAAGTACTP  
 651 TGAAGGACTG AAAACGAAC ATGATAGTAA TCCTGTATAA AAGACGTTC  
 701 CAATATGTAT CAAGCTCCTC ATAGAAAGTC TTACTGGAAA GTCCCTCTTA  
 751 CCCAAAACTC CTAGTACAAA GGAAGAAATG CAAAGCGGCT TATTATTATG  
 801 AAGTCTCTGC AAGACTTGTA AGCCGACTTG GGGAGGAGC ATAACAGAT  
 851 CTCCTTAAAC AGTCTATAGT ATAGCTAATG AAGGAGACAA TCAGCTCTCG  
 901 ATTTCGGTTC AAGAGTTTAA AGAACGAGAG CTGATGTCCA TCCAAGATGG  
 951 TGATGATGCT GAAGAGTATC GGTTCGCGG TCAGCAACAC GGTGAGCGTT  
 1001 ACACAGAGGC AATAGAACAA GTTCTACGAA ACAGTCTAGC AGCCAACTA  
 1051 CAATGGCATG TGATCAACAC TATGAATCTT TTCATGGGA AAAATCTCGG  
 1101 TCTAGTTACA GAACACCTAC AAGATACTCT CGGCGCTGTA ACTTTAGCT  
 1151 AAATACACAGT GGACACACAT CAAGGCAGAG AAGACGCTGA TTTGTCAGGT  
 1201 GCTCTTTTTC TAAATAAGTA TTTAAATCTT GGAAATCAAC TTGTTAATAG

1251 GGTCTTTAAA TCCATGCAAA AAGCAGATCC AGAACCRAA GCTTTAATCC  
 1301 GTGAGTTTGC TCTAGATATA TTATATGCAT CCTACGGCT TCCTCAAATC  
 1351 TCCGCTCATA CCGAGGCTCT TTCTACACTC TTAATGGACC CAGAGACCTA  
 1401 TGAACCTAAT AAGCTTGTGA TCGCTACTTT GCTCTATGTA TTAAAGATCA  
 1451 TCGAACTATA A

The PSORT algorithm predicts inner membrane (0.5989).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 139A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 139B) and for FACS analysis.

These experiments show that cp7288 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 140

The following *C. pneumoniae* protein (PID 4377359) was expressed <SEQ ID 279; cp7359>:

1 MPGSVSPPL SPVIVRERV SSSGSLIOP HAVLKISILI FALVTILGIV  
 51 LVVLSSALGA LPSLVLTVSG CIAIAVGLIG LGILVTRIL STIRKVDAMG  
 101 YDAAVKEBOY LSRIRLELESE NREIRDNRRA VEDQCAHLE ENKDLRDPY  
 151 LHGMTERLIA SLERINQALV AENILLKDWL ASLSRDFRAY KQKPLGLALE  
 201 PWKEDIACIM EQNLFLKPEC IANVKSLELE TORLEFLYPKG PQSLVNRFPAP  
 251 RSRFFQTPKY EYNSRNENED GKVAAVCARL KKEFFSAVLG ACSYEELGGI  
 301 CERAVALKET LPLPEAVYDT LVQEFNLLT ARLSKWECWF YSYPLRLPYL  
 351 SVDYCKRLFV QLPEELCLKL FTTGSPEDQA LVRLFSYVRN HIPAVLASTFG  
 401 LFPPEPTEGGSV FVLLPKQENL LNSQLEVLAT RYLRDTPVRN SEWTGSPFEMM  
 451 FSYNEMCKEI SEGRIRFARD YETRHSEEPF PSLSEEGEG BEFLPPCSEE  
 501 EVSVLERPDL DVDSMWVWHP PVPKPL\*

The cp7359 nucleotide sequence <SEQ ID 280> is:

1 ATGCCAGGTT CTGTGTCATC ACCTCCTTTG TCCTCTGTAA TTGTCGTGA  
 51 AAGGGTCCCA TCCTCTTCAG GATCCGACCT CATACAGCCT CATGCTGTGT  
 101 TAAAGATCTC CATCTTAATT TTTGCGCTTG TGACAATTTT AGGAATTTGT  
 151 CTTGTAGTGT GTCTAGTGC TTTAGGAGCT CTTCCTAGTT TAGTTTTGAC  
 201 GGTTCCTGGT TGTATTGCCA TAGCTGTAGG CTGATTTGGT TTAGGGATTC  
 251 TTGTGACACG GCTGATCTCT TCTACGATCA GAAAAGTAGA TGCCATGGGT  
 301 TATGATGCTG CGGTCAAAGA AGACGAGTAT TTGTACAGTA TCAGAGAATT  
 351 AGAGTCTGAA AATAGAGAGA TTAGAGATAG AATCTGTGCT GTCCAGATTC  
 401 AGTGTGCCCA TTTATCCGAA GAGACAAGG ACCTTAGGGA TCCCGAATAT  
 451 CTACATGGAA TGACTGAAAG GCTCATTCGC AGCTTAGAAA TAGAGATCA  
 501 AGCTCTCGTA GCTGAGAAC TTTCTCTCAA AGACTGGAAT GCAAGCCTAT  
 551 CTAGAGATTT CGCGCATAT AAGCAAAAT TTCTCTTGG GGCAATAGAA  
 601 CCCGGAAGAG AAGATATTGC ATGTATCATG GRACAAAATC TCTTTTAA  
 651 ACCGGAATGT ATCCGATGG TTAAGTCTCT TCCATTAGAG ACGCAACGGC  
 701 TGTTTTATATA TCCAAAAGGA TTTCACTCTT TAGTTAATCG ATTTGCTCCG  
 751 CGGTCTCGCT TTTTCCAGAC TCCAAAGTAT GRATATAACA GTAGGAATGA  
 801 AAATAGGAGC GGAAGGTTAG CCGCATGTGT CGCCCTTTG AAAAAAGAT  
 851 TCTTCACTGC TTTTATAGGA GCCTGTAGTT ACGAAGAACT AGGGGGCAT  
 901 TGTGAAAGAG CAGTAGCACT TAAAGAGACG TTGCATTGCG CTGAAGCTGT  
 951 CTATGATACC CTAGTTCAGG AGTTCGCCAA TCTCTTACT GCTGAGAGTT  
 1001 TATGGAAGA ATGTGCTCTT TATTTCTCAT CTACCTTCG TCCCTATCTT  
 1051 TCTGTGGATT ACTGTAAAG GTTATTGTGA CAACTTTTGT AGGAACCTCG  
 1101 CCTAAGCTTT TTATCAACCG GATCTCCAGA AGACCAAGCT TTGGTTCCGC  
 1151 TTTTCTCTTA CTATAGGAAT CATATTCCTC CAGTCTTGCG CTCATTGGT  
 1201 TTGCCCCCGC CTGAGACAGG GGGGCTGTGA TTTGTATTCG TACCAAACA  
 1251 AGAAGACCTT CTTTGGAGTC AAATTGAGGT GCTGGCTACA AGGTATCTCA  
 1301 AAGATACCTT CGTGAGAAAC TCAGAAATGGA CGGGCTCTTT CGAGATGATG  
 1351 TTTTCTTATA ACGAGATGTG TAAGGAGATC TCCGAAGGAA GGATCTGTT  
 1401 TGCTGAAGAC TATGAACAGA GGCAATTCGA AGAATTCCT CTTCCCTCTG  
 1451 TCTCTGAAGA AGGAGAGGGC GAAGAAATTC TTCTCCTG CTCTGAAGA  
 1501 GAGGTTTCGG TCTCTGAGCG CCCAGATCTA GATGTAGACT CTATGTGGGT  
 1551 CTGGCATCCG CCGGTCCCTA AGGGACCTCT TTA



The PSORT algorithm predicts inner membrane (0.7453).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 140A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 140B) and for FACS analysis.

- 5 These experiments show that cp7359 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 141

The following *C.pneumoniae* protein (PID 4377374) was expressed <SEQ ID 281; ep7374>:

10  
1 MDKSSGNSG CIWHPTQSA LDSTPIKIVR GEGAYLYAES GTRYLDAISS  
51 WWCNHLHGSH PYITKRLCBQ AQKLEHVIFA NPTHEPALEL VSKLAPLLPE  
101 GLERFFSSDN GSTSIRIAMK IAVQYYYNQN KAKSHFVGLS NAYHGDTFGA  
151 MSIAGTSPTT VPPHDLPLPS STIAAPYVGK EBLAIAQAKT VFSESNIAAF  
201 IYRPLLQGAG GMLMNYNPEGL KETILAKHYV GVLCIADEIL TFGPGRTPLEF  
251 ASEPTDIPPD IICLSKGLTG GYLPLALTVT TKEIHDAFVS QDRMKALHIG  
15 301 HTFTGNPLGC SAALASLDLT LSPKCLQQRQ MIERCHQREF EAHGSLWQRC  
351 EVLGTVLALD YPAEATGVFS QVRDHLNRFF LERGVLLRPL GNTLYVLPPY  
401 CIQBEDLRIT YSHLQDALCL QPQ\*

The cp7374 nucleotide sequence <SEQ ID 282> is:

1 ATGGACAAGC AATCATCAGG GAATTCAGGG TGTATCTGGC ACCCCTTCAC  
20 51 TCAATCTGCA TTAGATTCTA CACCCATAAA GATTGTAAAG GGAGAAGGTG  
101 CTTACCTCTA TCGGGAATCA GGAACAAGAT ATCTTGATGC GATATCTTCA  
151 TGGTGGTGCA ACCCTCCACGG TCATGGGCAT CCCTACATTA CAAAAAAATT  
201 ATGTGAGCAA GCACAGAAGT TAGAAGCTGT GATCTTGGCA AATTTCAACC  
25 251 ATGAACCGGC TCTAGAGCTC GTATCGAAAC TCGCTCCCTC CCTTCTGTGA  
301 GGTCTAGAAC GTTCTTTTTT CTCTGACAAC GGATCAACGT CTATCGAAAT  
351 AGCAATGAAG ATTGCTGTGC AATATTACTA CAATCAAAAC AAGGCTAAGA  
401 GCCATTTTGT TGGACTCAGC AATGCCATAT CACGAGATAT ATTTGAGGCT  
451 ATGTCGATAG CTGGACAGAG CCCTACTACA GTTCCCTTTC ATGATCTTPT  
501 TCTTCCTTCC AGTACAATGT CTGCTCCCTA TTATGGCAAG GAAGAGCTTG  
30 551 CCATGCGCCA AGCAAAAACA GTCTTTCTTG AAGCAATAT CGCAGCGTPT  
601 ATCTATGAGC CGCTATTGCA AGGTGCTGGA GGGATGTATA TGTATAATCC  
651 CGAAGGCCCTA AAGGAGATTC TCAAGCTTGC CAAGCATPAC GGGGTCTCTC  
701 GTATTGCTGA TGAATATCTT ACTGGCTTTG GCGCTACGGG TCCACTGTMT  
751 GCTTCTGAAT TTACAGACAT TCCTCTGTAG ATTATCTGTC TTTCTAAAGG  
35 801 TCTTACAGGA GGCTATCTCC CTCTAGCCTT GACAGTAACC ACTAAGAAAA  
851 TTTATGATGC CTTTGTCTCC CAAGATCGGA TGAAGGCATC GCTTCATGTC  
901 CATACCTTCA CAGGAAATCC TTTAGGCTGT AGTGTGCGCC TCGCTCTCTT  
951 GGATCTCACC CTATCTCCAG AATGCCATCA ACAAGGCCAA ATGATAGAAC  
1001 GGTGTCATCA AGAGTTTCAA GAAGCTCATG GTTCCCTATG GCAACGGTGT  
40 1051 GAGGTCTTGG GCACGGTACT CGCTCTAGAT TACCCTCGAG AAGCTACAGG  
1101 ATATTTTCCA CAATATAGAG ACCATCTCAA TCGCTTTTTC TTAGAAGCTG  
1151 GAGTCTCTCT TCGTCTCTTA GGGACACAC TGTATGTGCT GCCCCCTTAC  
1201 TGTATCCAAG AAGAAGATCT CCGGATTAAT TATTTCTACC TACAGGATGC  
1251 CCTATGCTCA CAACCAAGT AA

- 45 The PSORT algorithm predicts cytoplasm (0.2930).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 141A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 141B) and for FACS analysis.

These experiments show that cp7374 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

**Example 142**

The following *C.pneumoniae* protein (PID 4377377) was expressed <SEQ ID 283; cp7377>:

```

1 MREETVSWSL EDIREIYHTP VFELIHKANA ILRSNPLHSE LQTCYLISIK
5 51 TGGCVEDCAY CAQSSRYHTH VTPEPMKIV DVVERAKRAV ELGATRVCLG
101 AAWNRKDDRR YFDRVLAMVK SITDLGAEVC CALGMLSEBQ AKKLYDAGLY
151 AYNHNLDSPP EFYETIITTR SYEDRLNFLD VVNKSGISST CGGIVMGES
201 EEDRIKLLHV LATRDHIPES VFNLLNLFID GTPLQDQPP I SPFWEVLRTIA
251 TARVVFPRSM VRLAAGRAFL TVEGQTLCLPL AGANSIFYGD KLLTVENNDI
301 DEDAEMLKLL GLIIRPSPGI ERGNFCYANN S*

10 The cp7377 nucleotide sequence <SEQ ID 284> is:

1 ATGCGTGAAG AAACGTGATC CTGGTCATTG GAAGACATCC GCGAANTTTA
51 TCACACTCCC GTATTTTGAGC TGAATTCACAA AGCCAATGCC ATATTGCGTA
101 GTAATTTCCT CCATTGAGAA CTGCAGACTT GCTATCTGAT TTGATTAATA
15 151 ACTGGTGGAT GCGTTGARGA TTGCAGCTAC TGTGCCCCAAT CTTCGCGCTA
201 TCATACCCAC GTACACACAG AACCTATGAT GAAAATTGTA GACGTTGTGG
251 AAAGGGCAAA ACGTGCTGTA GAGCTAGCGC CCACCTCGTG GTGTCTTGGG
301 GCTGCTGGC GCAATGCTAA GGACGATCGA TACTTCTGATA GAGTCTCGCG
351 TATGGTGAAA AGTATCACAG ATCTCGGAGC CGAGGTTTGT TGTGCTTTAG
20 401 GCATGCTCTC CGAAGAGCAA GCTAAAAAAC TGTATGATGC AGGACTTTAT
451 GCCTACAATC ATAATTGATA CTCTTCTCCG GAATTCCTATG AAACATAAAT
501 CACAACACGT TCTTATGAAG ATCGCCTCAA CACTCTTGAT GTAGTAAATA
551 AATCTGGCAT TAGTACATGC TCGGIGGTA TTGTAGGTAT GGGAGAATCT
601 GAAGAAGACC GTATAAAGCT TCTTCATGTT CTTCGACAAA GAGATCATAT
651 CCCAGAAATC GTACCTGTAA ATTTACTTTG GCCGATTGAC GGCAGCCCTT
25 701 TGCAAGACCA GCGCTCGGAT TCTTCTGGG AAGCTTTCGG AACCATAGCA
751 ACGCGACGGG TTGTTTTCCTC CAGATCCATG GTACGACTTG CTCGAGSAGG
801 CGCTTCTCTC ACGATAGAAC AACCAACCTT ATGTTTCTTA GCGCGTGCCA
851 ACTCCATATT CTATGGAGAT AAACGTGTTGA CTGTAGAAAA CAATGATATA
901 GATGAAGATG CTGAATATGAT CAAACTTTTA GCGTTAATCC CTCGCCCTTC
30 951 ATTGTGAATA GAAAGAGGTA ACCCATGTTA TGCCACAAT TCCTAA

```

The PSORT algorithm predicts cytoplasm (0.2926).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 142A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 142B) and for FACS analysis.

- 35 These experiments show that cp7377 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

**Example 143**

The following *C.pneumoniae* protein (PID 4377407) was expressed <SEQ ID 285; cp7407>:

```

40 1 MCVPNNSWFR MCGNFNCERW EVITTEBETR QSASDISSEA GSSGGAAPIT
51 TQPTKITKVE KRVQFNPAQO DESTTHMIQE AGELVDSLLS HRRTOGCTEY
101 CYDSYATGCG QRCGSPGRLI CGTYXAOCLD REDNQVAGLV HECBQTHGPI
151 AVALAARTMG LNLMLVEKN TILSEEGKNE FRQHCSEAK QLYGTMQSLS
201 QNFFLBGVNS IRRGLDDSL VQVAFSFIAT RSWEKTISEE EASGTSASAN
251 STRIPACYLI NTSPLTTSRL SCGSRDARRP SSVGAEPPYV AKKYNDNGMA
45 301 RQLGKIQTIN LKGTGFSALG PFGLLIYKML NSFLLSASQS TBSILKHTGG
351 EICYTCNPNR DIVVLLMLAI GYCPANTDET SVVDIHMI DD PIMTIFYRLG
401 YSYRTGRKTS AFLKKKPSLV RQBSLDCMPT ABSVPLMSSL EEEDEDEDDDD
451 EDGNLAYQQR ILBCSGHLQT LFLGKINKE *

```

The cp7407 nucleotide sequence <SEQ ID 286> is:

```

50 1 ATGGTTTGCC CAAATAATTC TTGGTTCAGA ATGTGTGAAA ATTCAACTG
51 CGAATGGGTT GAAGTAACAA CAACAGAGA AACAACGCGC CAATCGGCTT
101 CAGATATAAG CGAAGAAGCT GGTTCGAGTG GAGGAGCTGC TCCTATAACT
151 ACGCAACCTA CTAAAAATTAC AAAAGTAGAG AAACGCTGTC AATTTAATAC

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201 TGCTCAAGGT GATGAAAGTA CAATACACAT GATCCAAGAA GCAGGAGAAT  
 251 TGGTAGACTC CMTCATATCA CATAGACGAA CGCAAGATG TACAGATAT  
 301 TGTATATGACA GTTACGCAAC TGGATGTGGT CAGCGTTGCG GATCTTTTGG  
 351 AAGACTCATTT GTTGGAACTG ATAAAGCGTG TTGCTTAGAC AGAGAGGATA  
 401 ATCAGGTTGCG TGGACTTGTC CATGAATGCG AACAGACCCA TGGTCTTATT  
 451 GCCGTTGCTTT TAGCTGTCTAA AACTATGGGC CTCACCTTAA TGGAACTTGT  
 501 AGAAAAAACC ACTATTTTGT CTGAAGAACA GAAAAATGAA TTTAGACAGC  
 551 ATTGCTCGGA AGCTAAAACC CAATCTTATG GAACGATGCA GAGCCTTTCT  
 601 CAAAACCTTT TCCTTGAAGG AGTCAACAGC ATTAGAGAAC CGCGCTTAGA  
 651 CGATTCTACTA GTCCAAAGCG TGTCAAGCTT TATGCTTACA AGGCTTGGGG  
 701 AAAAAACTAT AGAATCAGAG GAAGCCTCAG GAACATCTTC TGCTCTTAAT  
 751 TCTACACGCA TTCTTGGCGT CTATATCTTA AATACGAGCC CCTTAACGAC  
 801 GTCAACGCTTA TCCTTGGGAT CAAGAGATCG GCGACGCCCA TCTTCAGTGG  
 851 GTGCGAGAGCC CCAGTACGTA GCAAAAAAAT ACAATGACAA TGGCAATGGCC  
 901 AGACAATTAG GAAAAATCCA AGTCCAACAT CTAATAACAG GAGATTTTTC  
 951 AGCTTTAGGT CCTTTTGGTC TCCTGATTTG TGAATAATG AATGATCTTC  
 1001 TCTTATCTGCT ATCACAAGC ACATCTTCTA TTTCAAAACA CACAGGTGGA  
 1051 GAAATATGTT ATACGTGGCC AAATTTTCGT GATATCGTGC TTTTATTTAT  
 1101 GTTAGCGATT GGCTATTGCC CTGCMAATAC CGATGAGACA TCTGTCGTAT  
 1151 ATATACACAT GATAGATGAT CCGATTATGA CCACTCTTCA TCGACTACAA  
 1201 TACAGCTATA GAACAGGGAA AACTTCAGCA TCGTTTATTA AAAAGAAACC  
 1251 CTCATTAGTA AGACAGGAAA GTCTTGATTG TCCTACCCCT CGAGAATCTG  
 1301 TCCCTCTCAT GTCAAGTCTC GAAGAAGAA ATGAAATGAA AGATGATGAT  
 1351 GAGGATGGGA ATTGGCGGTA TCAACAGCGT ATCTCTGAAT GCTCGGGTCA  
 1401 TTTACAACT CTATTTTATG GATAAAAAT AAAAAGAAA TAA

The PSORT algorithm predicts inner membrane (0.1319).

The protein was expressed in *E. coli* and purified as a GST-fusion product (Figure 143A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 143B) and for FACS analysis.

- 30 These experiments show that cp7407 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone:

#### Example 144

The following *C. pneumoniae* protein (PID 4376432) was expressed <SEQ ID 287; cp6432>:

35 1 MTRSTIESSD SLCSRSFSQK LSVQILKNLC ESRLMKITSL VIAFLTLIVG  
 51 GALIALAGGG VLSPPLGLIL GSVLVLFSSI YLVSCCKFFT LKEMTMTCSV  
 101 KSKINIWFEK QRNKDIEKAL ENFDLFGENK RNVGNRSARN QLEMILHETD  
 151 GILKRYMKG AKMYFVL\*

The cp6432 nucleotide sequence <SEQ ID 288> is:

40 1 ATGACTAGAA GTACTATTGA AAGCAGTGAT TCGCTATGCT CAAGGTCCTT  
 51 TTCTCAAAAA TTAAGTGTC AGACATTAAG AAATCTCTGT GAAAGTAGAT  
 101 TAATGAAGAT CACTTCTCTT GTGATTCGCT TCCTAAGCTT AATTGTGGGG  
 151 GGTGCTCTTA TAGCTTTAGC AGGAGGGGGG GTTCTTCTCT TCCTCTTTGG  
 201 GCTAATCTTA GGAAGCGTAC TCGTTTGTGT TTCTTCTATC TATTTAGTCT  
 251 CTTGTTGTAA ATTTTTCATT TTAAGAAGA TGACAAATGAC CTGTAGTGTC  
 301 AARATCAAAA TCAATAATAT GTTTGAAAAG CAACGAACAA AAGAACTCGA  
 351 AARGGCMATTA GAGAATCCAG ATCTCTTTGG AGAAAAAAG AGAAATGTGT  
 401 GAATATCGTTC GCACAGAAT CACATAGAAA TGATCTTACA CGAGACTGAC  
 451 GGAATATTAT TGAAGAAGATA TATGAAAGGA GCTAAATAT ACTTTTATTT  
 501 ATGA

- 50 The PSORT algorithm predicts inner membrane (0.5394).

The protein was expressed in *E. coli* and purified as a his-tagged product (Figure 144A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 144B) and for FACS analysis.

These experiments show that cp6432 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 145

The following *C.pneumoniae* protein (PID 4376433) was expressed <SEQ ID 289; cp6433>:

```

5      1  MNWVFKTIDH  VDFESEIDIR  KVVSCYKLIK  EQQPEFRSLI  SELLGVIKRG
      51  LRLLRKSKYQ  EQARTVSEDE  APLFLCLTRSY  YQDGYLTPLR  AGPRDLINHY
     101  IHLRRRENPK  HFFSPKHPFY  YARLAFNFSV  CVYRELFDIE  RLTKMYVEGD
     151  YSRQEKENLQ  AILSFVKTL  EGKDFLIEHK  DTDLGRGFT  DVFTCT*
```

The cp6433 nucleotide sequence <SEQ ID 290> is:

```

10      1  ATGAATTGGG  TTCCAAAAAC  AATAGACCAT  GTAGATCCAG  AATCAGAGAT
      51  AGATATACGT  AAGTCGTCT  CCTGCTATAA  GTTGATAMAA  GAATGTC AAC
     101  CTGAATTCG  ATCTCTTATA  AGTGAATTAC  TAGGAGTGTAT  TCGGTGTGGC
     151  TTAAGACTAT  TAAAACGTC  TAAGTATCAA  GAACAGGCTA  GAACCTGTATC
     201  TGAATGAAGT  GCACCTCTTT  TCTGCCTGAC  TCGTCTCTAT  TATCAAGATG
     251  GTTATCTCAC  GCCATTAAAG  GCAGGACCTC  GTGATCTTAT  AAATCACTAT
     301  ATACACTTGC  GTCGCGGAGA  GAATCCTAAG  CATTTTTTCAT  GTCTTAAGCA
     351  TCCATGTTAT  TATGCTCGAT  TGGCTTTTAA  TGAGTCAGTG  TGTGCTCTATA
     401  GAGAACTCTT  TGATATAGAG  CGACTTACAA  AAATGTATGT  CGAGGGTGAT
     451  TATTCTAAG  AACAGAGAA  AAACCTACAG  GCTACTCTTA  GTTTTGTAAG
     501  AACTCTAGAT  GAAGAAAGG  ACTTCTTAT  TGAACATAAA  GATACCGCAT
     551  TCATTGGGAG  AGGTTTACT  GATGTGTCT  GCACTTAA
```

The PSORT algorithm predicts cytoplasm (0.4068).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 145A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 145B) and for FACS analysis.

These experiments show that cp6433 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 146

The following *C.pneumoniae* protein (PID 4376643) was expressed <SEQ ID 291; cp6643>:

```

30      1  MGYLPVSATD  VLFESPAAPL  INSANTONQK  LIELKGQQA  ESSPRITTSV
      51  ILEVLLVIGC  CLIVLSLLAI  RPAQLFTLET  GHFAAIAVLA  VSGTILLVAV
     101  IILPCFLAIV  PFAAKTKYKY  VKTVDDYASV  HSHQQTFTLG  TIFSGIVYAE
     151  SQAQL*
```

The cp6643 nucleotide sequence <SEQ ID 292> is:

```

35      1  ATGGGATATC  TTCCAGTATC  TGCTACGGAC  GTTCTTTTGG  AAAGTCCAGC
      51  CGCTCCCTTA  ATCAATAGCG  CAAACACACA  AAATCAGAAA  CTCATAGAAC
     101  TCAAGGGGAA  GCAGCAAGCT  GAGTCTTCTC  CACGGAACAT  CACTTCTGTG
     151  ATATTGGAAG  TCTCCTAGT  GATCGGATGC  TGCTCATATG  TTCTTAGTGT
     201  ATTGGCATT  CGCCCTGCTC  TGCATATCAC  TCTAGAAACT  GGACATCCAG
     251  CTGCCATTGC  AGTCCTTGCT  GTCTCAGGAA  CAATTCTATT  GGTGGCTGTT
     301  ATCATCTTGT  TTTGCTTTCT  AGCAGCTGTG  CCATTCTGCT  CTAAGAAAC
     351  TTAATAAAT  GTTAAGACGG  TTGATGACTA  TGCTTCTTGG  CATCTCATC
     401  AGCAAAACCC  GACCCTAGCG  ACTATCTTTT  CAGGTATCGT  CTATGCAGAA
     451  TCCACGGCGC  AATTATAG
```

45 The PSORT algorithm predicts inner membrane (0.6859).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 146A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 146B) and for FACS analysis.

These experiments show that cp6643 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 147

The following *C.pneumoniae* protein (PID 4376722) was expressed <SEQ ID 293; cp6722>:

```

1 VSSTLMGVFP SSLPEESADL FIINKEIVAL GEKGNVFLTH SIPMHIAAIT
51 ILVIVALAGI AIICLGCSYQ SILLIAGVIV LTILTLCLQ ALVGFIFIR
101 QLPQQLHTTV QFIREKIRPE SSLQLVNTAQ RKTQTDTLKL YEELCDLSQK
151 BFKLQSTLYQ KRFELSHKNE KTNQN*

```

The cp6722 nucleotide sequence <SEQ ID 294> is:

```

1 GTGTCTAGTA CTTTAAACGG GGTATTTCCC TCATCCCTTC CGGAAGAGTC
51 TGCTGATTTA TTCATTACGA ATAAGGAGAT CGTAGCTTTG GGGGAGAAGG
101 GCAATGTTTT TCTCACCAC TCCATTCCTA TGCATATTGC TGCGATTACG
151 ATCTAGTAGTA TTGTAGCTCT TGTGGAAATC GCTATTATCT GTTTGGGTTG
201 CTATAGCCAA AGCATCTCTT TGATTGCCGT TGGCATTTGT CTTACTATTT
251 TGACTCTCTC TCGCCTACAA GCCTTGGTAG GATTATTATA ATTCAATCCGG
301 CAGCTCCCTC AGCAGCTCCA TACGACAGTA CAATTTATCA GGGAGAAGAT
351 TCGACCTGAA TCCTCTCTAC AGCTTGTAAC CAATGCACAG AGAAAAACCA
401 CTCAGATATC GCTAAAGTAA TACGAAAGAC TCTGCGACCT CTCACAAAAA
451 GAGTTCAAAC TGCAATCAAC TCTTTATCAA AAACGTTTTG AGCTTTCTCA
501 CAAGAAATGAA AAGACAAATC AAAACTAG

```

The PSORT algorithm predicts inner membrane (0.6668).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 147A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 147B) and for FACS analysis.

These experiments show that cp6722 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 148

The following *C.pneumoniae* protein (PID 4377253) was expressed <SEQ ID 295; cp7253>:

```

1 MSBLAPCSTG LQMPVHTQVH HALDTRRVIL TIAACLSLIA GIVLVGLGAA
51 AILPSLFQVI GGMILILFSS IALIYLYKKT REVDQIALEP LPEMISKDQS
101 IIDFVKTRDY ASLEKKATFA YTHHYHYDGS MYFYREIPRF MLGSYLALRK
151 DMDRQALF*

```

The cp7253 nucleotide sequence <SEQ ID 296> is:

```

1 ATGAGCGGAC TCGCCCCCTG CTCGACAGAG TTGCAGATGG TCCCCCATAC
51 GCAGGTCCAT CATGCCCTTG ATACGCGGAG AGTCATTCTA ACGATAGCCG
101 CCTGTCTGTC TTAAATTGCA GGAAATCGTG TGGTTGGCTT AGGTGCTGCA
151 GCAATCTGTC CCTCGCTTTT TGGAGTCAIT GGAGGAATGA TTCTTATCTT
201 GTTTTCTTCG ATCGCCCTCA TTTATTATTA CAAGAAGACA AGGGAGGTGG
251 ATCAGATTGC TCTGAGCCTT CTTCCTGAGA TGATTTCATA AGATCAAAGC
301 ATTATAGATT TTGTAAGAGC ACGAGACTAT GCATCTTTAG AAAAGAAGCG
351 GACCTTTGCT TATATCTATA CTCATTATTA CGATGGAGAC ATGCTCTTCT
401 ATAGGGAGAT CCTAGATT TTGTTAGGCT CTTATCTCGC GCTTCGCAAA
451 GACATGGACC GCCAAGCTCT TTTTGA

```

The PSORT algorithm predicts inner membrane (0.5394).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 148A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 148B) and for FACS analysis.

These experiments show that cp7253 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 149

The following *C.pneumoniae* protein (PID 4376264) was expressed <SEQ ID 297; cp6264>:

```

1  VISGLLLFLV  RREVPVRSR  EIPRGVSVTP  SREPALEKAQ  KEPETKKILD
51  RLPKELDOLD  TYIQEVFACL  ERLKDPKYED  RGLLTEAKRK  LRVPDVVEKD
101 MMSFLLDIQR  VLNEEAYYVE  HCQDPLENIA  YELFSSQELR  DYICAGVCGY
151 LPSGDARADR  LKRSVKVMD  RFRMRTWKSW  BASVMDLSHY  GVARELFKKA
201 VGVLEESVYK  LLFKSYRDAF  YECEKAKIQR  DGRFKNL*

```

The cp6264 nucleotide sequence <SEQ ID 298> is:

```

1  GTGATTTCGG  GACTTCTATT  CTTCTAGTA  AGACGAGAG  TTCCGACAGT
51  ACGTTCAGAG  GAAATTCCTA  GAGGGGTTC  TGTGACCCCT  TCTGAAGAGC
101 CTGCTCTAGA  GAAGGCTCAA  AAGAACCGG  AGACAAAGAA  AATTTTAGAT
151 CGGTTGCCGA  AGGAATTGGA  TCAGTTAGAT  ACGTATATTC  AGGAAGTGTT
201 TGCATGTTTA  GAGAGGCTGA  AGGATCCTAA  GTACGAAGAT  CGAGGCTCTT
251 TAACAGAGGC  GAAGGAGAAA  CTTCCAGTTC  TTGACGTTGT  TGAGAAAGAT
301 ATGATGTCAG  AGTTTTTAGA  CATACAACGA  GTGTTGAATG  AGGAGACATA
351 TTATGTAGAA  CATTTGCAAG  ATCCCCTAGA  GAATATAGCC  TACGAGATTT
401 TCTCTTCCCA  AGAGCTTCGT  GATTACTACT  GTGCAGGGGT  GTGTGGGTAT
451 TTGCTTCTCG  GGGATGCTCG  AGCGGATCGA  TTAAGAGAGT  CAGTTAAGGA
501 GGTAAATGAT  CGCTTTATGA  GGGTGACCTG  GAAATCTTGG  GAGGCATCAG
551 TCAATGTTGA  TCATAGCTAT  GGGGTAGCGC  GAGAGTTAT  CAAGAAGGCA
601 GTAGGAGTAC  TAGGAGAGAG  TGTCTATAAA  ATTTCTGTTA  AGAGCTATAG
651 AGATGCGTTT  TATGAAATGT  AGAAGGCCAA  GATCCAGAGG  GATGGGCGTT
701 TCAAAATGTT  ATAG

```

The PSORT algorithm predicts cytoplasm (0.2817).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 149A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 149B) and for FACS analysis.

These experiments show that cp6264 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 150

The following *C.pneumoniae* protein (PID 4376266) was expressed <SEQ ID 299; cp6266>:

```

1  MLLLSISQALF  LTLGIPGLSA  AISFGLGTGL  SALGVLMIS  GLICLLVKRE
51  IPTVREBEIP  EOVSLAPSEE  PALQRAQKEL  AQLFKELDQL  DTDIQEVFAC
101 LRKLKDSKYE  SRSFINDAKK  ELRVDFVVE  DTLSEIFELR  QIVAOBOWDL
151 RFLNKGRLSL  MMTABESELD  LPHVSKRLSY  LPSGDVRGEG  LKSKAKETVA
201 RLMSLKEIHH  KVAVAFDRNS  YAMA EKAPAK  ALGALESBYK  RSLGQSYRDK
251 FLESERAKIP  WNHGHTWLRD  DAKSGCAEKK  LGMPRNVRGN  LGKQSPG*

```

The cp6266 nucleotide sequence <SEQ ID 300> is:

```

1  ATGCTCTTAC  TGATTTTCAG  AGCTCTCTTT  CTGACGTTAG  GGATTCACAG
51  ATTGAGTGCA  GCAATTTCTT  TTGGATTAGG  CATCGGTCTC  TCCGCTTAGT
101 GAGGAGTGCT  GATGATTTTC  GGACTACTAT  GTCTTTTAGT  AAACGAGAG
151 ATTCCGACAG  TACGACCAGA  AGAAATTCCT  GRAGGGGTTT  CCGTGGCTCC

```

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```

201 TTCTGAGGAG CCAGCTCTAC AGGCAGCTCA GAAGACTTTA GCTCAGCTGC
251 CTAAGGAATT GGATCAGTTA GATACAGATA TTCAGGAAGT GTTCGCATGT
301 TTAAGAAAGC TGAAGAGATTC TAAGTATGAA AGTCGAAGTT TTTTAAACGA
351 TGCTPAAGAA GAGCTTTCGAG TTTTTCGACTT TGTGGTTGAG GATACCTCTCT
401 CGAGATTTT CGAGTTGCGG CAGATTGTGG CTCGAAGAGGG ATGGGATTGA
451 AACCTTTTGA TCAATGGGGG ACGAAGCCTC ATGATGACTG CAGAATCTGA
501 ATCGCTTGA* TTGTTTCATG TATCGAAGCG GCTAGGCGAT TTACTTCTCG
551 GGGATGTTGCG AGGGGAGGGG TTAAGAAAT TCAGGAAGGA GATAGTGCGT
601 CGTTTGATGA GCTTGCATTG CAGAGATCAC AAGGTGGCGG TAGCGTTTGA
651 TAGQAATTCC TATGCGATGG CAGAAAAGGC GTTTGCGAAA CGGTTGGGAG
701 CTTTAGAAGA GAGTGTGAT CCGAGCTCTGA CGCAGAGTTA TAGAGATAAA
751 TTTTGGAGA GCGAGAAGCG GAAGATCCCA TGGAAATGGCG ATATAACCTG
801 GTTAGAGATG GATGCGAAGA GTGGCTGTGC TGAAGGAAGC CTCGGGATGC
851 CGAGGAACGT TGAAGAAAT TTAGGAAGC AGTCTTTTGG GTAG

```

15 The PSORT algorithm predicts inner membrane (0.3590).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 150A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 150) and for FACS analysis.

20 These experiments show that cp6266 is a surface-exposed and immunoaccessible protein and that they it is a useful immunogen. These properties are not evident from the sequence alone.

#### Example 151

The following *C.pneumoniae* protein (PID 4376895) was expressed <SEQ ID 301; cp6895>:

```

1 MKIKKSFQYS LCQAKRFQNM LPNHPDCLQ FVNLQKQDR LAYGELIILL
51 SKYQQCTFSS LLKERTCSLN RAKQHLLYKI LRDFNTMQLH RSLGLNGWGE
101 IFMSPLC*

```

The cp6895 nucleotide sequence <SEQ ID 302> is:

```

1 ATGAAGATTA AAAAATCTTT TCAATACAGT TTATGCCAAG CAAAGAGATT
51 TCAGAACATG CTGCCAAACC ACTTTGATCC ATGTTTCCAG CCAGTGAATT
101 TACAACCTCA ACAAAGACGA TTGGCATAAG GGGAGCTCAT CATATTGCTA
151 TCTAANTATC ACAAAGACAC CTTTCTCTCT TTGTTGAAGG AAGAAACATG
201 TTCTCTTAAT CGTGGCAAGC AGCACTTATT GTATAGAGAT TTGAGAGATT
251 TTAATACTAT GCAGCATCTA AGTCCCTCG GATTAAATGT TTGGGGAGAG
301 ATCCCTATGA GTCCTTGCCCT CTA

```

The PSORT algorithm predicts cytoplasm (0.3264).

35 The protein was expressed in *E.coli* and purified as a his-tag product (Figure 151A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 151B) and for FACS analysis.

These experiments show that cp6895 is a surface-exposed and immunoaccessible protein and that it is a useful immunogen. These properties are not evident from the sequence alone.

#### 40 Example 152 and Example 153

The following *C.pneumoniae* protein (PID 4376282) was expressed <SEQ ID 303; cp6282>:

```

1 MSLLNLPSSQ DSASEDSTSQ SQIFDPIRNR ELVSTPREKV RQRLLSFLMH
51 KLNYPKKLII TEKELKTI PP LLMRKGTLP KRPDILII PTPTTDAQGN
101 THNLGDPKPL LLEICKALAV NQNALEQLLS YNYSIGATCI AMAGKHSQVS
151 ALFNPKTQTL DFYPLPEYS QLNLYFISLN L*

```

45

The cp6282 nucleotide sequence <SEQ ID 304> is:

```

      1  ATGTCCTTAT  TGAACCTTCC  CTCACGCCAG  GATTCTGCAT  CTGAGGACTC
    51  CACATCGCAA  TCTCAAAATCT  TCGATCCCAT  TAGAAATCGG  GAGTTAGTTT
   101  CTACTCCCGA  AGAAAAAGTC  CGCCAAAGGT  TGCTCTCCTT  CCTAATGCAT
   151  AAGCTGAAC  ACCCTAAGAA  ACTCATCATC  ATAGAAAAAG  AACTCAAAAC
   201  TCTTTTCTCT  CTGCTTATGC  GTAAAGGAAC  CCTAATCCCA  AATCGCCTCC
   251  CAGATATTCT  CATCATCACT  CCCCCACAT  ACACAGACGC  ACAGGGAAAC
   301  ACTCACAAAC  TAGGCGACCC  AAAACCCCTG  CTACTTATCG  TATGTAAAGC
   351  CTTAGCCGTA  AACCAAAATG  CACTCAAAAC  ACTCCTTAGC  TATTAATCTC
   401  CTATCGGAGC  CACCTGCAT  GCTATGGCAG  GGAAACACTC  TCAGATGTCA
   451  GCTCTCTTCA  ATCCAAAAC  ACAAACCTCT  GATTTTATTC  CTGGCCTCCC
   501  AGAGTATTCC  CAACCTCTAA  ACTACTTTAT  TTCTTTAAAC  TTATAG
  
```

The PSORT algorithm predicts cytoplasm (0.362).

The following *C.pneumoniae* protein (PID 4377373) was also expressed <SEQ ID 305; cp7373>:

```

   1  MSTTIVKHFI  HTASRWEPVL  KEIVASNYMH  AQWINTLSFL  EMSGAKKISA
   51  SEHPTEVKKE  VLKHALEPR  HGHLKTKLIS  RISBTSPLPY  TSKNLLGGLL
  101  TKYVLHLLDL  RTCRVLENEY  SLSGQTLKTA  AVILVTYAIE  LRASELYPLY
  151  HDILKKAQSK  ITVKSTILEE  QGHLQEMERE  LKDLPHGHEK  LGYACQFEGE
  201  LCLQFVERLE  QMIFDPSSTF  TKF*
  
```

The cp7373 nucleotide sequence <SEQ ID 306> is:

```

      1  ATGCTACAA  CCACAGTAAA  ACACTTTATC  CACACAGCCT  CTCGTTGGGA
    51  GCCCGTTCTC  AAGAGATCG  TAGCTTCCAA  CTATTGGCAT  GCACAATGGA
   101  TAAATACCCCT  GTCTCTTTTA  GAAATAGTGT  GAGCAAAAAA  AATCTCCGCA
   151  AGTGAACATC  CTACGGAGGT  AAGGAGAGAA  GTTTTAAAC  AGTGTCTGTA
   201  AGAATTTCGT  CATGGTCACT  ATCTAATAAC  TCAGATTCTC  AGACTCTCAG
   251  AGACTTCTCT  CCTGACTACT  ACATCTAAAA  CTCAGTCGGG  GAGTACTGGA
   301  ACAAAATATT  ACCTCCATCT  TCTAGATTTA  AGSAGAGGCC  GCGTATATTT
   351  AAATGAATAC  TCCCATTCGG  GACAAACGTT  AAAAAGTGA  GCGTATATTT
   401  TAGTGACTTA  CGCAATCGAA  CTGCGTGGTT  CTGAACCTTA  TCCCTCTGTA
   451  CACGATATTC  TGAAGCAAGC  TCAAGTAA  ATAACGGTAA  AATCCATATT
   501  CTTAGAAAG  CAAAGCCATC  TGCAGAGAT  GGAACGTGAA  CTTAAAGATC
   551  TCCCCACGG  GAGAGCACT  TTAGCGTATG  CTTGCCAAAT  CGAAGGCGAG
   601  CTTTGCTTGC  AGTTTGTAGA  GAGATTAGAA  CAAATGATCT  TCGATCCTTC
   651  CTCGACTTTT  ACAAGTCTCT  AG
  
```

The PSORT algorithm predicts cytoplasm (0.1069).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 152A; 6282 = lanes 8 & 9; 7373 = lanes 2-4). The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 152B & 153) and for FACS analysis.

These experiments show that cp6282 & cp7373 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequence alone.

Example 154 ,  
 Example 155 ,  
 Example 156 ,  
 Example 157 and  
 Example 158

The following *C.pneumoniae* protein (PID 4376412) was expressed <SEQ ID 307; cp6412>:

```

      1  MSSSEVVFTQ  VHGLGFGGLS  SKSVVPFKKS  LSDAPRVVCS  ILVLTGLGLA
    51  LVCGIATTCW  CVPVGLMGG  ICAIVLGAIS  LALSFLPWLGG  LPSNCCGSKR
   101  VLFGEGLLRD  KLLDGGFSRA  APSGMGLPGD  GSFRASPTSC  LBEELQABIQA
   151  VTQAIQMSD  D*
  
```

The cp6412 nucleotide sequence <SEQ ID 308> is:



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1 ATGAGCAGTT CGGAAGTTGT TTCCAGACA GTTCATGGCC TTGGCTTTGG  
 5 1 TGGATTGTCT TCAAAAAGTG TTGTCCTTT TAAGAAAAGT CTTTCGGATG  
 101 CGCCCCGCTGT TGTGTGCTCG ATTTAGTTGT TGACTCTGGG CTTGGGAGCG  
 151 CTTGTGTGTG GTATTGCCAT TACTTGTGG TGCTCCCGG GAGTTATTTT  
 5 201 AATGGGGGGA ATTTGCGCTA TACTTTTAGG TGCAATPTCT TTAGCTTTAA  
 251 GTCTATTTTG GTTGTGGGOT TTATTTTCTA ATTGTGTGCG TTCTAAGAGA  
 301 GTTTTACCGG GTGAGGGGAT GCTACGGGAT AAGCTTTTAG ATGGTGGATT  
 351 TTCAAGAGCG GCACCTTCAG GAATGGGACT TCCGGGTGAT GGATCTCCAA  
 10 401 GAGCGTCAAC GCCATCTTGC CTAGAGGAAC TTCAAGACGA GATACAGGCA  
 451 GTTACTCAAG CTATCGATCA GATGTCAGT GATTGA

The PSORT algorithm predicts inner membrane (0.4864).

The following *C.pneumoniae* protein (PID 4376431) was also expressed <SEQ ID 309; cp6431>:

1 LRAGGSLVTT YPKRGQLRS PEQLRLVDDL VQSYFNHILHA IELDOGAIPQ  
 51 DLIGATYIIT FADFSTYILS LRSYQANSPS DDTWGIWFGS IDDPVQAVIS  
 15 101 FLKDHGFPAL STLAQDPLLC TNK\*

The cp6431 nucleotide sequence <SEQ ID 310> is:

1 TTGCGAGCAG GAGGTAGTCT TGTTAACACA TACCCCTAAGG AAGGTCAGAG  
 51 ATTGCGCTCC CCAGAACAGT TAAGAGTTCT GGATGATTTA GTGCAAGCT  
 101 ATCCAAATCA CCTACATGCG ATTTGAAGTTG ATTGTGCGTC AATCCCTCAA  
 20 151 GATTTGATCG GAGCACACTA TATCATCAGC TTCCGCGAGT TTTCACCTA  
 201 TATTCCTCTCT TAAAGAGCTC ACCAAGCCAA TTCTCCCTCC GATGATACAT  
 251 GGGGGATTGT GTTTGGATCT ATTGACGATC CTGTTCAAGC AGTCATMTCA  
 301 TTTTAAAG ATCATGGAAT TGCTCTTCCC TCGACCTTAG CTCAGATCC  
 351 TTTGCTTTGT ACTAACAGT AA

25 The PSORT algorithm predicts cytoplasm (0.2115).

The following *C.pneumoniae* protein (PID 4376443) was also expressed <SEQ ID 311; cp6443>:

1 MIMPTISNSP SPALNFELSL IPPTIVSSG TQTSLAYTIP AQRRSTLRI  
 51 ILDFIILILG LATIISTFIV IFFLNLNLL STPSIISSC LIIIVGLFLI  
 10 101 MGLYFMISSL DQGLVGLQK ELSQAEREE EYIQEIALR GAPRAESPTE  
 151 SPSTWL\*

The cp6443 nucleotide sequence <SEQ ID 312> is:

1 ATGATTATGA CTACTATATC TAACCTACCC TCCCCTGCAAT TGAATCCGGA  
 51 ACTTTCCTCT ATCTCCACCA CAACACTTGT ATCTTCAGGT ACGCAAACT  
 101 CTCATAGCTTA TACGATCCCC GCACAAGGAC GAAGATCCAC CCTACGTAT  
 35 151 ATATTAGATA TATTCATFAT CATCTCTGGT TAGCTACGCA TCATTCTTAC  
 201 CTTTATTTGT ATTTCTTTT TAAATGGGCT GAACCTGGCT TCGACCCCAT  
 251 CTATATATCTC TTCTCATGAT TTAATCATGT TTGGATGTCT TTTTGTGAT  
 301 ATGGGGTAT ATTTCATGAT CTCGAGTTTG GATCAGGGGC TTGTAGGCTC  
 40 351 TCTGCAAAAG GAATCTCTC AAGCCGAGA AAGAGAAGAA GAGTATATCC  
 401 AGGAATCGCA AGCTTTAAGA GGAGCTCTCA GAGCAGAAAT TCCCAAGAG  
 451 TCTCCTAGTA CCTGTTATG A

The PSORT algorithm predicts inner membrane (0.5585).

The following *C.pneumoniae* protein (PID 4376496) was also expressed <SEQ ID 313; cp6496>:

1 MLIGRYSDD QFTEATXNTP TIILGFVRD NLEGLTNFIS HIVSETSSSI  
 45 51 KDSVLRSLPI LGSILGCARL YSLSTNDPL DETQEKIWHF IFGALETGLL  
 101 GILLILFKII FVILHCFHL VIGFK\*

The cp6496 nucleotide sequence <SEQ ID 314> is:

1 ATGCTAATAG GCAGATACAG TAGTCATGAC CAATTCACGT AAGCACAAA  
 51 AAACACCCCA ACCATAATTA AGCTAGGTTT TGTTAGAGAT AATCTCGAGG  
 50 101 GATTAACGAA CCTATCTCT GAAATCGTCT CGGAACCTCT CTCTCTATT  
 151 AAAGATTCCG TTCTTCGCTC TCTTCTTAT TTAGGGTCAA TTTTAGGATG  
 201 CGCCCGACTT TACAGCACAC TCTCTACAAA TGATCCCTCT GACGAAACT  
 251 AAGAAAAGAT TTGGCACACT ATATTGGAG CTTTGAAGAA CTTAGGCTTA  
 301 GGGATCTCTA TCTCTTATT TAAATATAT TTTGTTATAT TACACTGCAT  
 55 351 ATTTCTATCTA GTTATTGGGT TCTGCAATA A

The PSORT algorithm predicts inner membrane (0.5989).

The following *C.pneumoniae* protein (PID 4376654) was also expressed <SEQ ID 315; cp6654>:

```

1 MKTKMNSRKK AGQNAIFNSP TPGVSSTLVL AWTWPWGYDK DVQDILERKD
51 FMSSSLSEKD SKEFLKNLFV DLENGPTSV HIHAZEAPTP LDHKGKPHFK
101 RDNVYLPGLK LGALNEAAVQ ANVSADTQFT LFLTQDECNP FHDKKRG*
```

The cp6654 nucleotide sequence <SEQ ID 316> is:

```

1 ATGAAACTA AAATGAAGCT TAGAAAAA GCAGGTCAAT GGGCAATTTT
51 CAATTCCTCCA ACTCCTGGTG TCAGTCAAC TTAGTTTTGA GCATGAGACTC
101 CTTGGGGTGA TTACAGCAAG GATGTACAAG ATATCTTTAGA AAGAAAGATG
151 CCGATGAGCT CTTGCGTTTC TGAAGAAAG TCAGAGGAGT TCTTGAAAAA
201 TCTGTTTGTG GATCTCTTTG AAAATGCGCTT CACATCAGTA CATATTACAG
251 CAGAAGAAGC TTTCACCTCT CTTGATCATA CCGGGAACCC TCACCTTTAAA
301 AGAGACAATG TGTACTTACC CGGAAAGTTG TTAGGCGCCT TGAATGAGGC
351 TGCGGTACAA GCCAATGTAA GTGCGGATAC TCAATTTACA TTGTTCTCTTA
151 401 CTCAGATGA GTGCAATCCT TTTCATGATA AGAAAGAGAG TTAA
```

The PSORT algorithm predicts cytoplasm (0.0730).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 154A; 6412 = lanes 2-3; 6431 = lanes 11-12; 6443 = lanes 5-6; 6496 = lanes 8-9; 6654 = lane 10; markers in lanes 1, 4, 7). The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 154B, 155, 156, 157 & 158) and for FACS analysis.

These experiments show that cp6412, cp6431, cp6443, cp6496 & cp6654 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from their sequences alone.

#### Example 159 and Example 160

The following *C.pneumoniae* protein (PID 4376477) was expressed <SEQ ID 317; cp6477>:

```

1 LLKFFLVCEE LCILTVATHR ALLETPLALS FFKELTKYV YRAKDILQLH
51 NYKGFITLNT SPLCS*
```

The cp6477 nucleotide sequence <SEQ ID 318> is:

```

1 TTGCTAAAGT TCTTTCTAGT ATGTGAAGAG TTATGTATAC TTACTGTTGC
51 TACACATAGA GCTCTCTTAG AAACCTCCTT AGCTCTATCA TTTTPTTAAAG
101 AACTTAAAGC AAAATATGTC TACAGGCGCA AAGACATACT ACAACTACAT
151 AACTATAAAG GATTACTAT CTTAATACA TCACCGTTAT GTTCTTAA
```

The PSORT algorithm predicts inner membrane (0.128).

The following *C.pneumoniae* protein (PID 4376435) was also expressed <SEQ ID 319; cp6435>:

```

1 LWSHFPRGFF MLFPCPTILL AKPFLNSEN YGLERLAATVD SYFDLQSQI
51 VFSLKQDQGI TVEELSAKDR KFKPGSMNCT LYTEDPILFA HNSFSCNSDI
101 QMRTPIPIH *
```

The cp6435 nucleotide sequence <SEQ ID 320> is:

```

1 TTGTGGTGGC ATTTCCTAAG AGGATTTTTT ATGCTCCCTT TTGCCCCTAC
51 CATCTCTCTT GCTAAACCTT TTTTAAATAG CGAGAATTAC GGCCTAGAAC
101 GTTTCAGCTGC AACCGTAGAT TCTTATTTTG ATCTGGGACA GTCTCAAAAT
151 GTCTTCTCTT CCAACACAGG TCAAGGAATC ACTGTGGGAG AATTGAGTGC
201 TAAAGATAGG AAATTCAGC CAGGCTCTAT GAACGTATCA CTGTACACTG
45 251 AAGATCCATT CTACCTGCT CATAATTCCT TTAGTAATTG CTCTGATATT
301 CAAATCGGTA CTCCGATTAG CCCTATACAT TAA
```

The PSORT algorithm predicts periplasmic space (0.4044).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 159A; 6435 = lanes 2-4; 6477 = lanes 5-7). The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 159B & 160) and for FACS analysis.

- 5 These experiments show that cp6477 & cp6435 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequences alone.

**Example 161 and  
Example 162 and  
Example 163**

- 10 The following *C.pneumoniae* protein (PID 4376441) was expressed <SEQ ID 321; cp6441>:

```

1  VEAGANVLVI DTAHAKSEGV FQTVLEIKSQ FQISLVVGN LVTAEEAVSL
51 AEIGVDVAVK GIGPGSICCT RIVSGVGYPQ ITAINTVAKA LKNSAVTVIA
101 DGRIRYSGDV VKALAAGADC VMLGSLLAGT DBAPGDIVSI DEKLFRKYRG
151 MGS LGAMKQG SADRYFQTCQ QKRLVPGVVE GLVAYKGSVH DVLQYLGGI
201 RSGMGVYGAB TLKDLKTRAS FVRLITESGRA ESHIHNIYKV QPTILNY

```

The cp6441 nucleotide sequence <SEQ ID 322> is:

```

1  GTGGAGCTG GAGCAATGT TCTAGTCATT GACACAGCTC ATGCACACTC
51 TAAGAGAGTA TTCCAAACAG TTTTAGAAT AAAATCCGAG TTCCACACAA
101 TTTCTTTAGT TGTAGGGAAT CTTGTACAG CTGAAGCCGC AGTTTCCCTTA
151 GCTGAGATTG GAGTTGACGC TGTAAAGGTA GGTATTGCGC CAGGATCTAT
201 CTGTACAACT AGAATCGTTT CAGGGGTCGG TTATCCACAA ATTACTGCCA
251 TTACAAACGT AGCAATCGTT CAGCGGTGAC TGTATTGCTC TACGACAGG
301 GATGGGAGAA TCCGCTATTC TGGAGGTGTG GTAAAGCAT TACGACAGG
351 AGCAGACTGT GTCACTGCTAG GAAGTTTGTCT TGCAGGACTC GATGAGCTC
401 CTGGGGATAT CPTTCTATTC GATGAGAGAG TTTTAAAG GACCGGGGC
451 ATGGGATCTT TAGGCGCTAT GAAACAAGGA AGTGCTGACC GGTATTTTCA
501 AACACAGGGA CAGAAAAGC TGGTTCCTGG GGGAGTTGAA GGAATAGTCG
551 CTTATAAAGG CTTCTTCCAC GATGTCTCTC ATCAAATTTT AGGAGGAATA
601 CGCTCAGGTA TGGGGTATGT TGGAGCTGAA ACTCTCAAAG ATTTAAAAAC
651 TAAGGCTTCC TTTGTTTCAA TTACTGTAAT TGGAGAGCTC GAAAGTCATA
701 TTCAATATAT TTCAAAAGTT CAACCAACCT TAAATTATTA A

```

The PSORT algorithm predicts bacterial inner membrane (0.132).

The following *C.pneumoniae* protein (PID 4376748) was also expressed <SEQ ID 323; cp6748>:

```

1  LPSBGTALNL FRIFAPLRNR VTTSYRARQ FDLHRIAIYV IGVLDSESSK
51 ILERLISYMS CIYESQMYL RPFMGKNVQ SAVLSKLHVE NLHRCGFSS
101 EDVAPSEPPF DLSIYVHTDR SCFLPTKKRS SSWELQTVEL FESIYPQSEP
151 LLMRPMLGS*

```

The cp6748 nucleotide sequence <SEQ ID 324> is:

```

1  TTGTTCTCTG AGGGGACAGC TCTAAATTTA TTTGCTATAT TTGCTCCACT
51 ACQCAACCGT GTGACTACAG AATACAGTCG TCGTAGACAA CCGGACCTAC
101 ATAGAATTGC CATCGTCTAT ATAGGAGTTC TCGATTCAAA AAGTTCGAAG
151 ATCCTAGAGC GCTTAATCTT TTATATGAGT TGTATCTATT CTGAATCCCA
201 AATGTATTTA AGATTCTTTA TGGGCAAGAA GATCTCTCCA ACTGCTGTAC
251 TCTCAAAATT ACATGTAGAA AATCTGCACA TCCGTTTGGG GTTTTTCAGC
451 GAGGATGCTG TCCAGAGAG TGAGCCCTTC GATCTCTCCA TCTAGGTGCA
351 CACAGATGCT AGCTGTCTCC TCCCTACGAA AAAACGAGC AGCTCCTGGG
401 AACTCCAAAC TTAGAAGCTC CAGAGTCRAA TATATCCACA GTGCGAATTC
451 CTATTGATGA GACCTCGAAT GCTTTCGTAG

```

The PSORT algorithm predicts cytoplasm (0.170).

- 50 The following *C.pneumoniae* protein (PID 4376881) was also expressed <SEQ ID 325; cp6881>:

-174-

1 MRPHRKHVSS KSLALKQSAS THVEITTRAP RLSPMLKQLI LEKSDHLPPM  
 51 ETIRVVLVTS KDKLGTVEVHV VASHGKEILQ TKVHNANPYT AVINAFKKIR  
 101 TMANKHSHNR KDRTKHDLGL AAKERLAIQ EEQEDRLSNE WLFVGLDAW  
 151 DSLTTLGVVP ASAKKKISKMS KMSIRLSQD RAIHQLESAA ENFLIFLNEQ  
 201 EHKIQCIYKK HDGNYVLIET SLKPGFCI\*

The cp6881 nucleotide sequence <SEQ ID 326> is:

1 ATGAGACCTC ATCGTAAACA CGTATCATCT AAAAGCTTAG CTTTAAAGCA  
 51 ATCTGCATCA ACICATGATG AGATCACAAC AAAAGCCCTT CGTCTCTCTA  
 101 TGGCCTCTAA ACAGCTGATC CTAGAGAAA GCGACCACTT CCCCCTATG  
 151 GAAACAATCC TTGTGTGTCT AACCTCTCAT AAGATATAGC TAGGCACGA  
 201 GGTGCATGTT GTAGCTTCTC ATGGCAAGA AATCCTTCAA ACTAAGGTTC  
 251 ATAACGCAA CCCATACACT GCAGTGTATCA ATGCTTTTAA GAAAAATCCG  
 301 ACCATGGCAA ATAAGCACTC CAATAACGT AAAGACAGGA CAAACATGA  
 351 TCTAGGTCTT GCAGCAAAAG AAGAACGTAT CGCAATACAG GAAGAACAAG  
 401 AAGATCGCTT TAGCAACGAG TGGCTTCCTG TCGAAGSCCT CGATGCCTGG  
 451 GATTCCTTAA AAACCTCTGG GTATGTTCCC GCATCAGCGA AAAAGAAGAT  
 501 CTCCAAGAAA AAGATGAGCA TTCGTATGCT ATCTCAAGAC GAGGCTATCC  
 551 GCCAGCTAGA GTCTGCCGCA GAAAACTTCC TGATCTTCTT GAACGAGCAA  
 601 GAGCATAAAA TCCATATGAT TTATAAAAA CATGACGGCA ACTATGTCTT  
 651 TATTGAACTT TCCCTCAAGC CAGGATCTCG CATCTGA

The PSORT algorithm predicts cytoplasm (0.249).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 161A; 6441= lanes 7-9; 6748 = lanes 2-3; 6881 = lanes 4-6). The recombinant protein was used to immunise mice, whose sera were used in Western blots (Figures 161B, 162 & 163) and for FACS analysis.

25 These experiments show that cp6441, cp6748 & cp6881 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequence alone.

#### Example 164 and

#### Example 165

#### 30 Example 166

The following *C.pneumoniae* protein (PID 4376444) was expressed <SEQ ID 327; cp6444>:

1 MEQPNVCIQD TTTVLVALNS FDPRLSDTH RLKQSPLEA ENALGEFTEG  
 51 LDTHSPLEEE VAIFILPGYH PKFYLSFIDR DDQGVHYEVL DGVPLKTVAA  
 101 CIIENSFLTD SMSPELLSEV KEALKR\*

35 The cp6444 nucleotide sequence <SEQ ID 328> is:

1 ATGGAGCAAC CCAATTTGTT GATTTCAGGAT ACTACAAGT TTTTGTATGC  
 51 CTTAATATAGC TTTGATCCTA GACTTAGTGA TGCACTCTAC AGACTTGGGA  
 101 AGCAATCAAC TCTTGAGAGCA GAAATAGCTC TTGGAGAAIT TATTGAAAGT  
 151 TTGGATACAA ATAGCTTTCC TTGAGAGGAA GTTGCAATT CATGCTGCC  
 40 AGGTATATCA CCTAAGTTTT ATTTATCTTT CATAGTAGG GAOGATCAAG  
 251 GTTGCACATA TGAAGTTTTA GATGCGTAT TTTTAAAGAC AGTCGCTGCT  
 301 TGTATATATAG AGAAGCTCCT CTTAAGTAT TTTATGAGCC CGGAGCTTCT  
 351 CAGCGAAGTT AAGGAAGCTC TGAACAGATG A

The PSORT algorithm predicts cytoplasm (0.2031).

45 The following *C.pneumoniae* protein (PID 4376413) was also expressed <SEQ ID 329; cp6413>:

1 MAVQSIKRAV TSAATSVGCV NCSREATPAF NTEERATSTA RSVIAAIIAV  
 51 VAISLLGLGL VVLAGCCPLG MAAGAITMLL GVALLAWAIL TILRLNLNIF  
 101 AEIIPSPGNNG EPNERNSATP FLBGGVAGSA GRGGGSPIQI LDLSAGAGS\*

The cp6413 nucleotide sequence <SEQ ID 330> is:

50 1 ATGGCTGTTC AATCTATAAA AGAAGCCGTA ACATCAGCCG CAACATCAGT

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51 AGGATGCTGA AACTGTTCTA GAGAGGCTAT ACCAGCATTT AATACAGAGG  
 101 AGAGAGCAAC GAGTATTGCT AGATCTGTTA TAGCAGCTAT CATGCTGTGT  
 151 GTAGCTATCT CTTTACTCGG ACTAGTCTTT GTATGTTCTG CTGGTGTGCTG  
 201 TCCCTTAGGA ATGGCTCGCG GGTCTATAAC AATGCTGCTG GGTGTAGCAT  
 251 TATTAGCTTG GGCAATACCTG ATTACTTTGA GACTGCTTAA TATACCTAAG  
 301 GCTGAAATAC GAGTCCAGG GAACAAGGT GAGCCTAATG AAGAGAAATC  
 351 AGCAACTCCT CCTCTAGAGG GTGGTGTGTC AGGAGAAGCC GGTGCGCGCG  
 401 GGGGTCACC TTTAACCCAA CTTGATCTCA ATTCAGGGCG GGAAGTTAG

The PSORT algorithm predicts inner membrane (0.6180).

10 The following *C.pneumoniae* protein (PID 4377391) was also expressed <SEQ ID 331; cp7391>:

1 MMLRVIELPL LPIKQALEKA FVQNSYKAK LTKVEPCFRE SPAYITSEER  
 51 LQSLDQTILER AYKEYQKRFP EPSRLESEVS GCREHLREQV KQFETQGDL  
 101 IKEELIFVSD VLFKRMVSL VSTVHVPFME FYVEYFELHR LRLRAQWMAN  
 151 AEIYSKVRKA FPEMLKETLE KAKAPREEY WLLCEERKSK EKRLILNKIE  
 201 AAQRVRKDL EPPIKETGKQ KRKKEYSFFI RLKS\*

The cp7391 nucleotide sequence <SEQ ID 332> is:

1 ATGATGCTTC GTGTCATAGA GCTTCCACTA CTTCTCATAA AGCAAGCGTT  
 51 GGAGAGAGCT TTGTACAAAT ATATATAGCTA CAAAGCGAAG TTAACCAAGG  
 101 TAGAAGCTTG CTTGACAGAG AGCCTGCTCT ATATACACTAG CGAAGAGCGA  
 151 CTCACAGAGTT TGGATCAGAC TTTAGACAGT GCCTACAAGG AGTACAGAA  
 201 GAGATTCAG GAGCCTTCAC GTTTGGAATC GAGATCAAGT GGATGTAGAG  
 251 AGCATCTTAG AGAGCAGGTA AACCAATTG AACTCAAGG ACTAGACTTG  
 301 ATCAAGAGAG AGCTTATATTT TGTATATGAT GTGTTATGCC GAAATATGTT  
 351 CAGTTGTCTA GTGTGCGACAG TCCATGTTCC CTTTATAGAG TTTTATTAIG  
 401 AGTATTTTGA GTGTCATAGA CTGAGGTTCG GGGCCCAATG GATGGCGAAT  
 451 GCGGAGATTT ATAGCAAAAT TAGAAAAGCA TTCCACAGAA TGTTGAAGGA  
 501 GACCTTAGAA AAAGCTAAGG CTCACAGAGA AGAAGAGTAT TGGTACTTCT  
 551 GCGAGGAGAG AAAGACTAAG GAGAAGCCTT TGAITCTCAA CAAGATAGAG  
 601 GCAGCTCAGC AGCGGCTAAA AGATTAGAA COTCTCCTTA TTAAGAGAC  
 651 AGGGAACACG AAACCGAAGA AAGAAATATC GTTTTTCATT CGATTAAAA  
 701 CGTGA

The PSORT algorithm predicts inner membrane (0.1489).

The proteins were expressed in *E.coli* and purified as his-tag and GST-fusion products (Figure 164A; 6444=lanes 11-12; 7391=lanes 2-3; 6413=lanes 4-6). The recombinant protein was used to immunise mice, whose sera were used in Western blots (Figures 164B, 165 & 166) and for FACS analysis.

These experiments show that cp6444, cp6413 & cp7391 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequence alone.

#### Example 167 , Example 168 , Example 169 and Example 170

The following *C.pneumoniae* protein (PID 4376463) was expressed <SEQ ID 333; cp6463>:

1 MKKKVTIDEA LKBIKLLEGA ATQEELCAKL LAQGFAATQS SVSRWLRIQ  
 51 AVKVAGERGA RYSLPSSSTK TTRHLVLSI RHNASLIVIR TVPGSASWIA  
 101 ALLDQGLKDE ILGTLAGDDT IFVTPIDEGR LPLLMVSIAH LGQVFLD\*

The cp6463 nucleotide sequence <SEQ ID 334> is:

1 ATGAAAAAAA AAGTAACTAT AGATGAGGCT TTAAGAGAAA TTTCACGTCT  
 51 TGAAGGAGCG GCAACTCAGG AGGAATATATG TGCAAAACATC TTAGCTCAAG  
 101 GTTTTGCTAC AACCCAGTCG TCTGTATCTC GTTGGCTACG AAAGATTACG  
 151 GCTGTAAAGG TTGCTGGAGA GCGTGGTGCCT CGTTATCTCT TACCCTCTTC

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201 AACAGAGAAG ACCACGACCC GTCATTGGT GCTCTCTATT CGCCATAACG  
 251 CCTCTCTTAT TGTAAATTCGT ACGGTTCCTG GTTCAGCTTC TTGGATCGCT  
 301 GCTTTGTTAG ATCAAGGSCCT CAAAGATGAA ATTCTTGGAA CTTTGGCAGG  
 351 AGATGACACG ATTTTTTGTCA CTCCTATAGA TGAAGGAGG CTCCTATTGT  
 5 401 TGATGSTTTC GATTGCAAT TTACTGCAAG TTTTCTTGGG TTAA

The PSORT algorithm predicts inner membrane (0.1510).

The following *C.pneumoniae* protein (PID 4376540) was also expressed <SEQ ID 335; cp6540>:

1 MSQQSSSTS TWEMKSFVP NVENPTPLPS PIPSEDEFIL AYEFPVLPKT  
 51 DPENAGANPP GTSTPNVENG IDLNLPLLGQ PNEQNANNP GTSGSNPTSL  
 101 PAPERLPETE ENSQEEBQGS QNNEDLIG\*

The cp6540 nucleotide sequence <SEQ ID 336> is:

1 ATGTCTCAAT GTCAGAGTAG CAGTACATCT ACCTGGGAAT GGATGAAATC  
 51 TTTTGTGCCA AACTTGAAGA ATCCAACTCC CCCCTTATCT CCTATACCTT  
 101 CTGAGGACGA ATTTATATTA GATACAGAGC CATTTGTCTCT ACCGAAAACA  
 151 GATCCAGAAA ACGCACAAAG TAATCTCCCA GGACATCTTA CACCGAATGT  
 201 AGAAACGGG ATCGATGATC TCAACCTCTCT TCTGGGCGAA CCCAACGAAC  
 251 AAAACAATGC CACAAATCCA GGAACCTCTG GATCTAATCC TACATCTCTA  
 301 CCCGCCCCCG AACGATCCCT TGAAGTGAAG GAGAACAGCC AAGAGAAGA  
 351 ACAAGGATCT CAAAATAATG AGGATCTTAT AGGATAA

20 The PSORT algorithm predicts cytoplasm (0.3086).

The following *C.pneumoniae* protein (PID 4376743) was also expressed <SEQ ID 337; cp6743>:

1 LREEGSVSFR EYFRAYMCDK IVAQKNFLPT LDAVIRQAGW RSQEKLNLFY  
 51 VESQALGREI KVSLEEYIQS MVGILGSLRT KKSFKFSVDV TPLEQALQER  
 101 CSSDDDEDAT ATSTATGATA SPTDMHDE\*

25 The cp6743 nucleotide sequence <SEQ ID 338> is:

1 TTGAGAGAGG AAGGTAGTGT TTCTTTTCTAGA GAATATTCTCA GAGCCTATAT  
 51 GTGTGATATA ATCGTGGCAC AGAAGAACTT CTTATTACTT TTAGACGCTG  
 101 TAATTAAACA GGCCGGTTGG AGATCACAAG AGAAACTCAA TTTATTATAT  
 151 GTTGAAAGTC AGGCTTTAGG AGAGAGAAATC AAGTCAAGCT TAGAGGAATA  
 201 TATTTCAGAGT ATGGTCGGGA TTTTGGGATC TCAGAGAAC AAGAAAAGCT  
 251 TTAAGTTTTC TGTCGAATCT ACCCTTTTAG ACAGGCGCTC ACAAGAAAGT  
 301 TGCTCTTCTG ATGATGACGA AGATGACACA GCAACTTCGA CCGCTACAGG  
 351 GGCAACAGCA TCTCCGACTG ACATGACAGA AGATGAGTAA

The PSORT algorithm predicts cytoplasm (0.2769).

35 The following *C.pneumoniae* protein (PID 4377041) was also expressed <SEQ ID 339; cp7041>:

1 MMLMLMIIG ITGSSGAGKT TLTQNIKEIF GEDVSVICD NYKIDRSHYT  
 51 PEERANLWD HPDAPNDLL ISDIKRLKNN EIVQAPVDF VLGNRSKTEI  
 101 ETIYPSKVIL VEGILVFENQ ELRLMDIRI FVDTDADERI LRRMVRDQVE  
 151 QGDSVDCIMS RYLSMVKPMH EKFIETPRKY ADIIVHGNR QNVVITNLSQ  
 40 201 KIKNHLENAL ESDETYMYVN SK\*

The cp7041 nucleotide sequence <SEQ ID 340> is:

1 ATGTTGATGA TGCTTATGAT GATTATTGGA ATTACAGGAG GTTCTGGAGC  
 51 TGGGAAAACC ACCCTAAACC AAAACATTAA AGAAATTTTC GGTGAGGATG  
 101 TGAGTGTATCT CTGCCAAGAT AATTATTACA AAGATAGATC TCATTATATC  
 151 CCTGAAGAAC GTGCCAATTT AATTGGGATC CATCCGAGCC CTTTGTATTA  
 201 TGACTTATTA ATTTCAAGCA TAAAAAGTCT AAAAAATAAT GAGATTGTCC  
 251 AAGCCCGAGT TTTTGATTTT GTTTTAGGTA ATCGATCTAA AACGGAGATA  
 301 GAAACGATCT ATCCATCTAA AGTATTCTT GTGAAGGTA TTTGTGCTCT  
 351 TGAAATCAAA GAACTTAGAG ATCTTATGGA TATTAGGATC TTTGTAGACA  
 40 401 CCGATGCTGA TGAAGAGATA CTACGCGGTA TGGTTGAGGA TGTTCAGAAA  
 451 CAAGGAGATA GCGTGGAGCT CATCATGTCT CGTTATCTTT CTATGGTAAA  
 501 GCCTATGCAT GAGAAATTTA TAGAGCCGAC TCGGAAATAT GCTGATATCA  
 551 TTGTACATGG AATTTACCGA CAAAACGTAG TAAACAATAT TTTGTACAG  
 601 AAAATTAATA ATCAATTTAGA GAAATGCCCTG GAAAGCGATG AGACGTATTA  
 55 651 TATGGTCAAC TCTAAGTAA

The PSORT algorithm predicts inner membrane (0.1022).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 167A; 6463 = lanes 2-4; 6540 = lanes 5-7; 6743 = lanes 8-9; 7041 = lanes 10-11). The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 167B, 168, 169 & 170) and for FACS analysis.

These experiments show that cp6463, cp6540, cp6743 & cp7041 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequence alone.

#### Example 171 and

#### Example 172 and

#### Example 173

The following *C.pneumoniae* protein (PID 4376632) was expressed <SEQ ID 341; cp6632>:

```

1  VQLFYQNMNES GWUWLCDFDS QEGFQLSRL VGLLHSSWAL YEAKBQFYLP
51  EVSLLTWBEL IEMQLLSKPT KHGVAKDLGN VPEKHFORPR QYLGSLDLNQ
101 RFENTFLNYP KYHLDR*
```

The cp6632 nucleotide sequence <SEQ ID 342> is:

```

1  GTGCAATATAT TTCAATATAT GAATGAGTCC GGATGGGATT GGCTTTGTGA
51  TTTTGATTCTT CAGGCGAGG GATTCCAGTT ATCACGCTGT GTTGGGCTGT
101 TACATTCGTC CTGGGCATTA TACGAGCAAA AAGAGCAATT TTACCTTCCT
151 GAGGTTTCTC TATTGACCTG GGAAGAACTG ATAGAATGCG AGTTATTAG
201 CAAACCAACA AAGCAGGGG TTGCAAAAGA TCTTTGTAAT GTATTGAAA
251 AACACTTTCA AAGGTTTAGA CAGTACCTAG GTTCCTTAGA TCTAATCAA
301 AGGTTGGAAG ATACCTTCTT GAATATACCT AATACCATT TAGATAGGA
351 GTGA
```

The PSORT algorithm predicts cytoplasm (0.3627).

The following *C.pneumoniae* protein (PID 4376648) was also expressed <SEQ ID 343; cp6648>:

```

1  MPVSSAPLPT SHRPSSONIG LMEPNKALK AKHQDKTTKT IKLLVKILVA
51  ILVIEVLGII AAFPITGTFP ICTIILGLLI LTTVLCLVLI VIKLALVNKT
101 EGTTAEQQIK RKLSSKSSIS*
```

The cp6648 nucleotide sequence <SEQ ID 344> is:

```

1  ATGCGCGTGT CCTCAGCCCC CTTACCCACA AGCCACCGCC CTTCCTCTGG
51  AAATCTAGGC CTATCGGAAC CAATATCCAA AGCTCTAAAA GCAAGGCATC
101 AAGATAAACC GACGAAGACG ATTAACCTTT TAGTTAAAAA CTTTGTTCGC
151 ATTCTAGTAA TAGAAGTTTT AGGAATTAAT GCACCTTTCT TTATTCCTGG
201 GACTCTCTCC ATCTGCTTGA TTACTCTTAG AGCCCTTATC CTTACAACAG
251 TACACTGTGT GCTTCTTCTT GTTATAAAGC TTGCCCTTGT AAACAAACC
301 GAAGGAACAA CTGCTGAACA GCAGATAAAA CGTAACCTCT CTTCTAAAG
351 TATTTCCTAG
```

The PSORT algorithm predicts inner membrane (0.6074).

The following *C.pneumoniae* protein (PID 4376497) was also expressed <SEQ ID 345; cp6497>:

```

1  MKPNSIIIFLE NTKHYDIFR EGFVRDRHGL MEASDWLLST EITTIIRSLG
51  AIPILGNILG AGRLYSVVYT SDEIMKQVY *
```

The cp6497 nucleotide sequence <SEQ ID 346> is:

```

1  ATGAAGCCAA ATAGTATPAT TTTTATTAGAA AATACTAAGC ATTATCCCGA
51  CATCTTTCTGA GAAGGATTAG TTGCTGATCG TCATGGACTA ATGGAAGCCT
101 CGGATTG9TT ACTTTCACAG GAAATTACGA TCATTGCTCT CATCTGGGAA
151 GCTATCCCTA TTTTAGGAAA TATCTTTGGA GCCGAGCAGC TCTATAGCGT
```

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201 TTGGTATACA AGTGCAGAG ATTGGAAAA ACAAGTGGTT TGA

The PSORT algorithm predicts inner membrane (0.145).

The proteins were expressed in *E.coli* and purified as his-tag products (Figure 171A; 6632 = lanes 5-7; 6648 = lanes 8-10; 6497 = lanes 2-4). The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 171B, 172, 173) and for FACS analysis.

These experiments show that cp6632, cp6648 and cp6497 are surface-exposed and immunoaccessible proteins and that they are useful immunogens. These properties are not evident from the sequence alone.

**Example 174 ,  
Example 175 ,  
Example 176 ,  
Example 177 and  
Example 178**

The following *C.pneumoniae* protein (PID 4377200) was expressed <SEQ ID 347; cp7200>:

1 MPFVIDNSSR NLQEVPESLD DLEQHAEESP THQSABESSL QLSLASSAIS  
51 SRVQLQLSLV LGMENSDPSS LRDVPFISAI YESSHTFPVP TPLVGVGVIN  
101 GSQSGYVDTP RESLHLSQLL GSRREVVVYN QGNFMEASLL NLCPRRPRRD  
151 PSPISLALLE LWEAFFLEHP PGSTFNPIFF W\*

The cp7200 nucleotide sequence <SEQ ID 348> is:

1 ATGCCCGTTC CTATAGATAA TTCCCTCTCGC AACCTACAAG AAGTTCCAGA  
51 AAGCCTAGAA GACCTCGAAC AACACGCAGA AGAATCTCCT ACTCATCAAA  
101 GTGCAGAAAG CAGTTCTTTG CAACCTGTCTC TAGCCTCCTC AGCAATTTCT  
151 AGTAGAGTAG ACAAACATATC TTCCCTCGTC TTAGGAATGG AAAATTCAGA  
201 TTTCTCCTCT TTAGAGAGAG TTCCCTATCTT CTCAGCTATC TACGAATCTT  
251 CAACACACAC ACCTGTCCCC ACTCCTCTAG TTGGCGTGGG ATATATCAAC  
301 GGAAGTCAAT CAGGATACTA CGATACACAA AGAGAATCTC TTCACTCAG  
351 CCAATTGTTA GGAAGCCGAA GAGTTGAAGT TGCTATAAC CAAGGAATC  
401 TCATGGAGGC CTCTTTGCTA AATCTGTGCC CCAGAAAGCC TCAGAGAGAT  
451 CCCTCTCCAA TTCTTTTAGC TCTATTAGAG CTCTGGGAAG CATTTTTTTT  
501 AGAACACCCC CCAGGTAGCA CTTTATATCC AATATTTTTT TGSTAA

The PSORT algorithm predicts cytoplasm (0.3672).

The following *C.pneumoniae* protein (PID 4377235) was also expressed <SEQ ID 349; cp7235>:

1 LNFVSTLTGS DFYAPVLEKI ERAFADTTGQ VILFSSSPDF IVHPIAQQLG  
51 ISSWYASCYR QDSABQTIYK KCLTGDKKAQ ILSYIKKINQ ARSHPTSDHI  
101 LDLPFLMLGE RKTVVPRQGR LKMKAKYYW NIV\*

The cp7235 nucleotide sequence <SEQ ID 350> is:

1 TTGAATTTTG TATCGACTCT GACCGGCTCC GATTTTATG CTCCTGTTTT  
51 AGAAAACTA GAAGNAGCTT TTGCAGATAC CACAGGACAG GTGATCCTTTT  
101 TTTCTCTTTC TCCAGACTTT ATTGTCCACC CCATAGCGCA GCAACTCGGG  
151 ATTAGTCTCT GGTATGCGTC GTGTTATCGC GATCAGTCTG CAGAACAGAC  
201 GATCTATAAA AATGTCTTCA CAGGGGATAA AAAAGCGCAA ATTTTGAGTT  
251 ATATTAAAAA AATTAATCAA GCAAGAGGCC ATACCTCTCT CGACCATATT  
301 TTAGATCTTC CTTTCTTATG GCTGGGAGAA GAGAAAACCG TCGTTCGCC  
351 TCAGGACGCA CTCAGAAAAA TGSCAAAAAA ATATTACTGG AATATCGTTT  
401 AA

The PSORT algorithm predicts cytoplasm (0.3214).

The following *C.pneumoniae* protein (PID 4377268) was also expressed <SEQ ID 351; cp7268>:

1 MMHRYFIPLL ALLIFSPSLV RAELOPSENK KGWPTQLSC AEGSQLCKFK



51 BAAYNNAIEE GKPGILVFFS BRPTPEFADL TNGSFSLSSTP IAKGFNVVVL  
101 CPGLISPLDF HKMDPVILY MGSFLEMPFE VBAVSGFRLC YILLDBQGGGA  
151 QQQAVLPLET KN\*

The cp7268 nucleotide sequence <SEQ ID 352> is:

5 1 ATGATGCACC GTTATTTTAT TCCTTTATTA GCACCTCTCA TTTTCTCTCC  
51 TTCCTTAGTC AGGGCAGAGC TACAACCAAG TGAAACACAGA AAAGGGGGGT  
101 GGCCTACACA ACTTTCTCTT GCAGAAAGGT CGCAACTCTT CTGTAAATTC  
151 GAAGCTGCCT ATAATAATGC AATTGAGGAA GGGAAACCTG GGATTTTAGT  
10 201 CTTTTTCTCT GAGCGACCCA CACCAAGATT TGCCGACCTA ACGAATGGTT  
251 CATTTTCTCT CTCTAGCCCA ATCGCCAAAG GCTTTAATGT CGTTGTGTTA  
301 TCGCCCGGGC TTATCAGTCC CTTAGACCTT TTCCACAAAA TGGATCCTGT  
351 GATTCTCTAT ATGGGAAGTT TTCTAGAGAT GTTCCCTGAA GTGGAGCCAG  
401 TTAGTGGCCC TCGCTTATGT TATATCTTAA TAGATGAACA GGGTGGGGCT  
451 CAATGTCAGG CTGTCTGCC TTTAGAAACA AAGAATTAG

15 The PSORT algorithm predicts inner membrane (0.1235).

The following *C.pneumoniae* protein (PID 4377375) was also expressed <SEQ ID 353; cp7375>:

1 MQRILIVGID TGVGKTIUSA ILARALNAEY WKPIQAGNLE NSDSNIVHEL  
51 SGAYCHPEAY RLHKPLSPHK AADIDNVISIE BSHICAFKTT SNLIETSGG  
101 PLSPTCTSKRL QGDVSSWSC SMILVSGAYL GSTNHTCLTV RAMRSRLNWI  
20 151 LGMVNVGYPE DEBHMLTQEL KLFITGTLAK EKEITKTIIS CYAEQNKRWV  
201 TSNHGGIQGV SGTFSLNHL\*

The cp7375 nucleotide sequence <SEQ ID 354> is:

1 ATGCAACGTA TCATCATTGT AGGAATCGAC ACTGCGCTAG GAAAAACCAT  
51 TGTCACTGCT ATCCTTGCTA GAGCACTTAA CGCAGAATAT TGGAAACCTA  
25 101 TACAAGCAGG GAATCTAGAA AATTGAGATA GCAATATTGT TCATGAGCTA  
151 TCGGGAGCCT ACTGTCATCC CGAAGCTTAT CGATTGCTATA AGCCCTTGTC  
201 TCCACACAA GAGCGCAAA TCATAATGT AAGTATCGAA GAGAOTCAT  
251 TTTGTGCCC AAAAACAACT TCGAATCTGA TTAATTGAGAC TTCGAGGAGA  
30 301 TTTTATATCC CCTGCACATC AAAAAGACTT CAGGGAGATG TGTTTTCTTC  
351 TTGCTCATGT TCTTGGATT TTAGTGAGCA AGCATATCTC GGAAGTATCA  
401 ATCACACCTG TTTAACTGTA GAAGCAATGC GCTCACGAAA CCTCAATATC  
451 TTAGGTATGG TGGTAAATGG GTATCCAGAG CACGAAGAGC ACTGGCTAAC  
50 501 TCAGAAATC AAGCTTCTTA TATCGGAGC TCTTGCCCAAG GAAAAAGAAA  
551 TCACAAAGAC ATCATTAAGC TGTTATGCGG AACAAATGAA GGAAGTATGG  
35 601 ACAAGCAATC ATCAGGGAAT TCAGGGGTGA TCTGGCACC CTTCACCTCA  
651 TCTGCATTAG

The PSORT algorithm predicts cytoplasm (0.0049).

The following *C.pneumoniae* protein (PID 4377388) was also expressed <SEQ ID 355; cp7388>:

1 MQVLLSPQLP PPPQHSVGS I SSPSLRLVLA ITFLVFGMLL LISGALFLTL  
40 51 GTPGLSAAIS FGLGLLSAL GGLMISGLL CLLVKREIPT VRPEIPEGV  
101 SLAPSEEPAL QAAQCTLAQL PKELDQLDID IQEVEFACLRK LKDSKYESRS  
151 FLNDAKKELR VFDFVVDTL SEIFELRQIV AQEGWDLNPL INGGRSIMMT  
201 AESSESLDLFH VSKRLGYLPS GDVRGEGGLKK SAKETVARLM SLHCBIHKVA  
45 251 VAFDRNSYAM AEKAFKALG ALSESVYRSL TQSVRDKFLE SERAKI PWNG  
301 HITWLDDAK SGCAEKKLRD AEERWKKFRK AVFVWDEDDG FDIINLLGDW  
351 GTVLDPYRQE RMDEITPHEL YKTTFLKRL HRKCALAKIT FEKKRSKKNL  
401 QAVESANARR LKYVRIMDYQ EFKAGERLE KLIALYFEVS VSIRENKIQE  
451 TRSNLEKAYE AIEENYRCCV REQEDYKKEE EKREARFRER GNKILSPREL  
50 501 BSSLBQFDHG LKNFSEKMLE LBGHLLKLQK EATAEVENKI LSDAESRLLEI  
551 VFEDVKEMPC RIEEIEKTLR MAELPLLPK KAFKACASQV NSCAEMLEKV  
601 KPYCKESLAY VTSKERLVSL DEBLRRAYTE CQKRFQDSDG LSESVRACRE  
651 QLRERTQEFQ TQGLDLVEKE LLVCSSRLRN TBCDCVSGVK KEAPPKGFY  
701 AQYYDEIYRV RVQSRMWTMS ERLRGGVQAC NKMLKAGLSE EDKVLKEBEY  
751 WLYREERKNK EKRLVGTQIV ATQQRVAAFE STEVPPIPEA PEEKPSLLDK  
55 801 ARSLFTREDH T

The cp7388 nucleotide sequence <SEQ ID 356> is:

1 ATGCAAGTAC TTCTATCTCC GCAGCTACCC CCCCCCCCC AACACTCTGT  
51 AGGGTCGATT TCTTCTCAT CTAAACTTCG CGTTTAGGCG ATTACTTTT

101 TAGTFTTTGG TATGCTCTTA CTGATTTTCAG GAGCTCTCTT TCTGACGTTA  
 151 GGGATTCACG GATTGAGTGC AGCAATTCTT TTTGGATTAG GCATCGGTCT  
 201 CTCGCCATTA GGAGGAGTGC TGATGATTTC GGGACTACTA TGCTCTTTTAG  
 251 TAAACACAGA GATTCCGACA GTACGACCAG AAGAANTTCC TGAAGGGGTT  
 301 TCGCTGGCTC CTTCGTGAGG GGCAGCTCTA CAGGCGAGTC AGAAGACTTT  
 351 AGCTCAGCTG CCTAAGGAAT TGGATCAGTT AGATACAGAT ATTCAGGAAG  
 401 TGTTCGCATG TTTAAGAAAG CTGAAAGATT CTAAGTAGGA AAGTCGAAGT  
 451 TTTTAAACAG ATGCTAAGAA GGAGCTTCGA GTTTTGTACT TTGTGGTTGA  
 501 GGAATACCCCT CCGGAGATT T TCGAGTTGCG GCAGATTGTG CCGTCAAGGG  
 551 GATGGGATTT AAACCTTTTG ATCAATGGGG GACGAGCCCT CATGATGACT  
 601 GCAGAACTCG AATCGCTTGA TTGTGTTTCA GTATCGAAGC GGCTAGGGTA  
 651 TTTACCTTCT GCGGATGTTC GAGGGGAGGG GTTAAAGAAA TCTCGAAGG  
 701 AGATAGTTCG TCGTTTGTAG AGCTTGTCAAT GCGAGATTCA CAAGTGTGGC  
 751 GTAGCTTTTG ATAGGAATTC CTATGCGATG GCAGAAAGGG CGTTTGCAGAA  
 801 AGCGTTGGGA GCTTTAGAAG AGAGTGTGTG TCGAGTCTG ACAGAGAGTT  
 851 ATAGAGATAA ATTTTGTGAG AGCGAGAGGG CGAAGATCCC ATGGAATGGG  
 901 CATATAACCT GGTAAAGAGA TGATCGGAAG AGTGGGTGTG CTGAAAAGAA  
 951 GCTTCGGGAT GCCGAGGAAC GTTGGGAAGAA ATTTAGSAAA GCAGCTTTTT  
 1001 GGGTGAAGA AGACGGGGGC TTTGACATCA ATAATCTCCT TGGAGACTGG  
 1051 GGGACAGTGC TTGATCCCTTA TAGACAAGAG AGAATGGACG AGATAACGTT  
 1101 CCATGAGTTG TATGAAGAAA CTACGTTTTT GAAAGAAGCT CACAGAAAGT  
 1151 GTGCGTTAGC GAAACANCC TTTGAAAGA AGAGATCTAA AAGAATTTG  
 1201 CAGGCGAGTC AGGAGGCGAA TGCACGTAGG TTGAATATG TAAGGGGATTG  
 1251 GTATGATCAG GAGTTTCAGA AAGCAGGGGA GAGATTGAG AAACGCTATG  
 1301 CTTTGTATCC TGAGGTTTCA GTCTCTATAA GAGAGAACAA AATACGAAG  
 1351 ACGCGCTCTA ATTTAGAGAA AGCCTATGAG GCTATCGAAG AGAATATCG  
 1401 TTGCTGTGTC CGAGAGCAG AGGACTACTG GAAAGAAGAA GAGAAGAGGG  
 1451 AAGCGGAGTT TAGGAGGAGG GGAACACAGA TTCTTCTCC TGGAGAGCTG  
 1501 GAAAGTCTTT TGGAGCAATT CGACCATGGT TTGAAAATTT TTCTGAGAAA  
 1551 ATTAATGGAA TTGGAAAGGC ATATCTTAAA ACTTCAGAAA GAAGCCACAG  
 1601 CAGAGTGGGA GAATAAATA CTTTCAGATG CAGAGAGCCG CCTTGAAGAT  
 1651 GTATTTGAAG ATGTCAAGGA GATGCCCTGT CGAATTGAGG AGATAGAGAA  
 1701 GAGCTTGCCT ATGGCGGAGC TGCCCTTACT TCCTCAAGAG AAGGCGTTTG  
 1751 AGAAGGCGCT CTCACAATAT AATAGCTGCG CAGAGATTGT GGAGAAAGTG  
 1801 AAGCCTTACT GCAAGGAGAG CCTCGCTAT GTGACTAGCA AAGAGCGTTT  
 1851 AGTGAGCTTG GATGAAGATT TACGACGAGC CTACACAGAG TGTCACAGA  
 1901 GATTCAGAGG GAATTCGGGT TTGGAGTCGG AAGTAAGAGC CTGTGAGAG  
 1951 CAACCTGCGAG AGCGGATCCA AGAGTTTGA ACTCAAGGGC TGGACTTGGT  
 2001 GGAAGAAAGAG TTGCTTTGTG TGAGTAGTAG ATTAAGAAAT ACAGAGTGGC  
 2051 ATTGTATATC TGGTGTAAAG AAGAAAGCAC CTCTGGTAA GAAATTTTAT  
 2101 GOCAGATATT ATGATGAGAT TTATCGAGTT AGAATTCAAT CCCGATGGAT  
 2151 GACGATGTCT GAGAGATTGA GAGAGGGAGT TCAAGCATGC AACCAAGATGT  
 2201 TGAAGGCAGG CCTAAGCGAA GAAGATAAGG TTCTTAAAGA AGAAGATAT  
 2251 TGGTTGTATC GAGAGGAGAG AAGAAATAAA GAGAAACGTT TGGTTGGTAC  
 2301 TAAAGATAGTA GCAACGACAG AGCGAGTTGC AGCATTTGAA TCCATAGTAG  
 2351 TTCCTGAGAT TCCTGAGGCC CCAGAGGAGA AACCGAGTTT GCTGGATAAA  
 2401 GCGCTTCTT TATTTACTCG CGAGACCAT ACCTAG

The PSORT algorithm predicts inner membrane (0.461).

The proteins were expressed in *E. coli* and purified as his-tag products (Figure 174: 7200=lanes 2-3;  
 7236=lanes 4-5; 7268=lanes 6-8; 7375=lanes 9-10; 7388=lanes 11-12). The recombinant proteins  
 were used to immunise mice, whose sera were used in Western blots (Figures 174, 175, 176, 177 &  
 178) and for FACS analysis.

These experiments show that cp7200, cp7235, cp7268, cp7375 & cp7388 are surface-exposed and  
 immunoaccessible proteins and that they are useful immunogens. These properties are not evident  
 from the sequence alone.

#### Example 179

The following *C. pneumoniae* protein (PID 4376723) was expressed <SEQ ID 357; cp6723>:

1 NATSVAPSPV PESSPLSHAT EVLNLPNAYI TQPHPIPAAP WETFRSKLST  
 51 KHTLCFAL/TL LLTLGGTISA GYAGYTNMI ICGILGLIIV L/TLILALLLA  
 101 IPLKKNQGTG LKIDBISQDI SSIGSGFVQR YGLMFSTIKS VHLPELTQTN  
 151 QEKTRILNEI EAKKESIQND ELKITECQNK LAQKQPKRS SQKSPMRISK  
 201 HLSKNPVLFP DC\*

The cp6723 nucleotide sequence <SEQ ID 358> is:

1 ATGGCAACTT CCGTAGCCCC ATCACCAGTC CCCGAGAGCA GCCCTCTCTC  
 51 TCAATGCTACA GAAGTTCTCA ATCTTCCTAA TGCTATATIT AGCGACGCTC  
 101 ATCCGATATCC AGCGGCTCCT TGGGAGACCT TTCGCTCCAA ACTTTCACCA  
 151 AAGCATACGC TCTGTTTTTC CTTAAACCTA CTGTAACTT TAGGGGGAAC  
 201 GATCTCAGCA GGTACGACG GATATACTGG AAACCTGAGTC ATCTGTGGCA  
 251 TCGGCTTGGG AATTATCGTA CTCACACTGA TTCTTGCTCT TCTCTAGCA  
 301 ATCCCTCTTA AAAATAAGCA GACAGAAACA AAACCTGATG ATGAGATATC  
 351 TCAAGACATT TCCTCTATAG GATCAGGATT TGTTCCAGAGA TACGGGTTGA  
 401 TGTCTCTTAC AATTAAGAGC GTGCTCTTTC CAGAGCTGAC AACACAAAAT  
 451 CAAGAAAAAA CAAGAATTTT AATGAAATTT GAAGCAAAA AGGAATTCGAT  
 501 CCAAAATCTT GAGCTTAAAA TTACTGAGTG CCAAAACAAG TTAGCACAGA  
 551 AACAGCCGAA ACGGAAATCA TCTCAGAAAT CATTATGCG TAGTATTAAG  
 601 CACCTCTCCA AGAACCTGT AATTGTGCTC GATTGCTGA

The PSORT algorithm predicts inner membrane (0.6095).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 179A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 179B) and for FACS analysis.

These experiments show that cp6723 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 180

The following *C.pneumoniae* protein (PID 4376749) was expressed <SEQ ID 359; ep6749>:

1 MSYYFSLNYL KVQHFQAAF DFTRSLCSRI SNFALGVIAL LPIIGQLYVG  
 51 LZWLLSRIKK PEPFSDVDQI VRVEHVVGHD HRSRVEDILK QRSLSEPRD  
 101 EGKVHGDLPF APFF\*

The cp6749 nucleotide sequence <SEQ ID 360> is:

1 ATGAGTTATT ACTTTTCTCT TTGGTATCTG AAGCTGCAAC AGCACTTTCA  
 51 AGCAGCAATT GATTTTACTC GTCCTCCGTG TTCACGAATT TCTAATTTTG  
 101 CTTTGAGGAGT GATTGCAATG CTCTCTATTA TTGGCCAGTT GTATGTAGGG  
 151 CTGAGCTGCG TCCTCTCTAG GATAAAAAG CCAGAAATTC CTTCGAGTGT  
 201 GGATCAGATC GTGCGAGTAG AACACGTCTG GGGTCACGAC CATAGAAGTC  
 251 GAGTTGAAGA TATTCTAAG AGACAAGGC TCTCATTAGA GCCTAGAGAC  
 301 GAGGGGAAGG TTCACGAGA TCTGCTTCA GCTCCTTTTT TTTGA

The PSORT algorithm predicts inner membrane (0.2996).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 180A). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 180B) and for FACS analysis.

These experiments show that cp6749 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

Example 181 ,  
 Example 182 ,  
 Example 183 ,  
 Example 184 and  
 Example 185

The following *C.pneumoniae* protein (PID 4376301) was expressed <SEQ ID 361; cp6301>:

```

1  LNQDLQNVYQ  ECQKATGLBS  EVSAYRDHLR  BQITEFPTQG  LDVIKEELLF
51  VSFPLSKSLK  YDFLIADIPC  MKFYEEYDG  IDKARVQSRW  LEKSEYRKA
101  KRGFQEMLKE  GLFKEDQALK  KAEYRLREK  RMNKEKLLIC  NKIEAAQQRV
151  QRFGPSDS*
```

The cp6301 nucleotide sequence <SEQ ID 362> is:

```

1  TTGAATCAGG  ATTTACAAAA  TGTATACCAA  GAGTCCAGA  AGGCTACAGG
51  TTTAGAAATCG  GAAGTGAGTG  CATATAGAGA  TCATCTTAGA  GAGCAGATCA
101  CAGAGTTTGA  AACTCAAGGG  CTGGACGTGA  TAAAAGAAAG  ACTTCTTTTT
151  GTGAGTAGTA  CTCTCAAAAG  TAAATTGAGC  TATGATCCAT  TAATAGCAGA
201  CATTCCTCGT  ATGAAGTTTT  ATGAGGAGTA  TTATGATGGC  ATTGATAAAG
251  CGAGAGTTCA  ATCCCGATGG  CTGGAGAAAT  CTGAGAGGTA  TAGAAAGCGC
301  AAGAAGGGAT  TCCAAGAGAT  GCTGAAGGAA  GGCCATTATCA  AAGAAGATCA
351  GGCTTTGAAA  AAAGCAGAGT  ATAGATTACT  TCGAGAGAAG  AGAATGAATA
401  AGGAGAAGCT  TTTGATTGTC  AATAAGATAG  AAGCAGCTCA  GCAGCGAGTC
451  CAAGAATTG  GACCCTCGGA  TTCATAA
```

The PSORT algorithm predicts cytoplasm (0.4621).

The following *C.pneumoniae* protein (PID 4376558) was also expressed <SEQ ID 363; cp6558>:

```

1  MNIPAPQVPV  IDEPVMVNTS  SYGLSLKSSL  RPITYLILAI  LAIATLMSVL
51  VPCCILISVGT  FVLCMLPLSL  VCSVLCAVYL  FYQQSSIEKT  KVFSITSPSV
101  PFSDEDLNLL  LQREEDSVSA  IDELLKNFFA  DDFRFRKMLP  YSNFLDEQQR
151  PMSREEDSH  TSKIL*
```

The cp6558 nucleotide sequence <SEQ ID 364> is:

```

1  ATGAACATAC  CCGCTCCCCA  AGTACCAGTC  ATAGATGAGC  CTGTAGTGAA
51  CAACACAAGT  AGCTATGGTC  TTTTATTGAA  AAGTATGTTA  AGACCAGATTA
101  CTTATTTGAT  TTAGCTATAT  TTAGCTATAG  CCACACTGAT  GTCTGTCTCT
151  TACTTTTGTG  GCATCAITAG  TGTGTGGGAG  TTTGTTTGG  GCATGCTGAT
201  CCTCTATCG  GTCTGCTCTG  TTCTTTGGGT  TGCTTATTTA  TTCTATCAGC
251  AATCTTCTAT  AGAAAAGACT  AAGGTCTTTT  CTATAACCA  TCTTTCAGTA
301  TTTTCTCTGT  ATGAGGATCT  TAATTACTCT  TTAGGTCGAG  AAGAAGATTG
351  AGTGTCTGCA  ATTGATGAAC  TTCTTAAGAA  CTTTCCAGCT  GATGATTTC
401  GTAGGCCGAA  GATGCTTCTT  TATTCAAATT  TTCTAGATGA  GCAGGGAAGG
451  CCTAATGAGA  GTAGGGAAGA  AGACTTCTAT  ACTTCCAAGA  TCTTATAA
```

The PSORT algorithm predicts inner membrane (0.4630).

The following *C.pneumoniae* protein (PID 4376630) was also expressed <SEQ ID 365; cp6630>:

```

1  MSMTIIVPHAL  FKNHCECHST  PFLSSRTIVR  IAIASLPCIG  ALAALGCLAP
51  PVSIVTGVSVL  AFIAFVILLS  VIALIFGEK  KLPTPTRIIP  DRPHTVIDEA
101  YGLSISAFVR  PQQVTLAEFR  QFSTALLCN  SPEEKIKQLP  SBLASKVESF
151  GISRLAGDLB  KNNWPFEDL  LSQCTPLYWL  QKFIISAGDQ  VCRDLGVFRE
45  201  CYGVYVLGFL  GYSTAKATIF  CKETHILLQ  LKEDVLLIK  NKALQEKMDT
251  DEVKAVIERI  VYTTVARGTL  KTRAGALKTK  TISKELLILLS  LHOYSFDQLQ
301  LITQLPRDAW  DWLCFVDMST  AYNLQCALV  GALSSQMLLD  ESSIDPDVNL
351  GLVYIQDLKE  AVQAFSASDE  PKELGKFL  RHLSSVSKRL  ESVLRQGLHR
401  IALEHGNARA  RVYDVMFTG  ARIHRKTSIF  FKD*
```

The cp6630 nucleotide sequence <SEQ ID 366> is:

```

1  ATGAGCATGA  CGATCGTTCC  ACATGCTTTA  TTTAAAAATC  ATTGCGAGTG
51  TCATTCTACC  TTCTCTTTGA  GTTCAAGGAC  TATTGTGAAG  ATAGCCAITG
101  CCAGCCTCTT  TTGTATAGGT  GCATTAGCAG  CTTTAGGCTG  TTTGGCTCCT
151  CCGGTTTCTT  ATATTGTTTG  GAGTGTTTTA  GCTTTTATTG  CCTTTGTGAT
55  201  TCTTTCTTTA  GTAAITTTAG  CTTTGTATTT  TGAGAGAAG  AAGCTCCAC
```

251	CAACACCAAG	AATCATTCCT	GATAGATTTA	CTCACGTGAT	AGATGAAGCT
301	TATGGCCCTT	CAATCTCTCG	ATTTGTGAAGA	GAACAGCAGG	TAACATTAGC
351	CGAGTTTAGA	CAATTTTCTA	CTGCCCTGTT	GTGTAAACATA	TCTCCTGAAG
401	AGAAATCAA	ACATTTGCTT	TCTGAATTCG	GAAGTAAATG	AGAGAGTTT
451	GGTATTAGCA	GGCTGCGAGG	TGATTTAGAA	AAGAATAAT	GGCCAATATT
501	TGAAGATCTT	TAAAGCCAAA	CCTGCCCGTT	ATATTGGCTT	CAGAAATTTA
551	TATCAGCAGG	AGATCCACAA	GTTTGTAGAG	ACCTAGGTGT	CCTTAGAGAA
601	TGTTTATGGT	ACTATTGGCT	AGGGCCCTTG	GGATACAGTA	CAGTAAAGGC
651	TACAATTTTT	TGTAAGAGAGA	CGCATCATAT	TCTTCAACAA	TTAACGAAGG
701	AGGAGCTTCT	TTTATPAAAA	AACAAGGCTC	TTCAAGAGAA	ATGGGATACT
751	GATGAAGTCA	AAGCAATTGT	AGAGCGTATC	TACACTACCT	ATACGGCAGG
801	AGGAACCTTA	AAGAOCGAAG	CAGGGGACTC	TACAAAAGAG	ACAACTAGTA
851	AGGAATTGCT	ATTGTTGAGC	TGTCATGGCT	ATTCTTTTGA	TCAGCTACAG
901	CTGATCACTC	AACCTCCTAG	AGATGCTTGG	GATTGGCTGT	GTTTGTAGAG
951	TAAACAGTACC	GCATACAACC	TTCAGCTTTG	TGCTCTTGTA	GGAGCTTTGT
1001	CATCCCAAAA	TCTCTCTGAG	GAATCTTCTA	TCGATTTTGA	TGTAACCTTA
1051	GGCCTGTATG	TGATTCTCAGG	TCTAAAAGAA	GCTGTTCAAG	CATTTTCTGC
1101	TTCTTGATGAG	CCAAAGAAAG	ACTAGGTAA	ATTCTTGTTA	AGGCATTTGA
1151	GTTTCAGTTT	TAGCGAGTAA	GAGAGTGTA	TAAGACAGGG	TCTTCACAGA
1201	ATAGCTCTAG	AGCATGGAAA	TGCCAGAGCT	AGGGTTTATG	ACGTCAATTT
1251	TGTAACAGGA	GCTAGAAATC	ATGACAGAGC	GAGTATCTCT	TTTAAGAGCT
1301	AA				

The PSORT algorithm predicts inner membrane (0.7092).

The following *C.pneumoniae* protein (PID 4376633) was also expressed <SEQ ID 367; cp6633>:

25	1	MVNIQPVYRN	TQVNYSQATQ	FSVCQPALSL	IIVSVVAANL	AIVALVCSQS
	51	LLSIELGTAL	VIVSLILFAS	AMFMYIKMRQ	EPKELLIPKK	IMELIQRHYF
	101	SIVVDVIRDO	EVSLYELIHL	ISLNLKTNVP	DKAPVYLQEK	LIQPGIEKFK
	151	DVHPSKLPNF	KEILLQHCPL	RHWLGRVLYPM	VSDVPTGYG	YYWCGPLGY
	201	ENAPSLPFERR	SLLLKKISF	GFALLDEGL	KKNTWSSSEL	VQIRQNLPTR
30	251	YYADKEEVDE	ARLNADYEQF	DSLHLILFSH	KLS*	

The cp6633 nucleotide sequence <SEQ ID 368> is:

35	1	ATGGGTTAATA	TACAGCCGTG	GTATAGGAAT	ACCCAAGTCA	ACTATAGTCA
	51	GGCTACCCAA	TTTTCGGTGT	GCCAGCCAGC	GCTTAGCGCTG	ATTATCGTTT
	101	CTGTTGTGTG	TGCTGTACTC	GCTATTGTAG	CTTTGGTATG	CAGTCAATCT
	151	CTTTATATCCA	TAGAGTTAGG	AACATGCTTT	GTTCATGTTT	CTCTATATCT
	201	TTTTCGCTTCT	GCTATGTTTA	TGATTATATA	GATGAGACAA	GAACCTAAGG
	251	AGTTGCTGAT	CCCTAAGAAA	ATCATGGAAC	TCATCCAAGA	ACATTTATCCA
	301	AGTATTGTGT	TGTGATTTAT	TAGAGATCAG	GAGGTTTCCA	TTTATGAGAT
	351	ACATCACTTG	ATCTCTATTC	TAAATAAGAC	GAATGTTTTC	GACAAAGCAC
40	401	CAGTATATTT	ACAAAGAAAA	CTCTTACAGT	TTGGCATPTGA	GAGGTTCAAA
	451	GATGTACATC	CAAGTAAGCT	CCCTAATTTT	GAGAAATATC	TTTCTACAGA
	501	TTGCCCATG	CATTTGGTTG	GACGTCCTGT	ATATCCCATG	GTATCGGATG
	551	TCATCCACAG	AACCTATGGA	TACTATTGGT	GTGGTCTCTT	AGGACTGTAC
	601	GAGAACGCTC	CCTCTCTTTT	TGAACGTOGA	TCTCTCTCAT	TGTTAAAGAA
45	651	AATTAGCTTT	GGAGAGTTTG	CTCTTTTAGA	AGATGGCTTC	AAGAAAACAA
	701	CGTGAGATT	TTTGGAACTC	GTTCAATCA	GACAAAACCT	TTTTCACAGA
	751	TATTTAGCTG	ATAAAGAAGA	GGTAGATGAA	CAGAGAGTAA	ACGCTGATTA
	801	CGAACAGTTT	GATTCCCTCC	TTCACCTTAT	TTTTTCTCAC	AAGCTCTCTT
	851	GA				

The PSORT algorithm predicts inner membrane (0.7283).

The following *C.pneumoniae* protein (PID 4376642) was also expressed <SEQ ID 369; cp6642>:

55	1	MATISPISLT	VDHPLVDTKK	KSCSNFPIKIQ	SRILLITAFI	AVLVTIGTLL
	51	IQLLLNIPVI	YFLTGISFIA	VVLSNPILYK	RATTLKPKRA	CGKHRIKPK
	101	RVSTNLQYSS	ISIAINRSKE	NWHEOPKDLQ	NLPAASALLT	DNPYIEIWKAK
	151	HSLFLSVSL	PGGNPEHLII	SAENSLGKTL	LIBETSNQAP	ISSVVDPTTS
	201	PKSLNLEAIQ	BTRVEINTEL	PAGDSGERLY	WQDFGRFRVY	LPQIPTTPEA
	251	IYQYVALYV	TYQTAINTN	TQIIQIPLY	LREHLYSREL	PPQSRMQQSL
	301	AMITAVKYMA	BLHPEYPLTI	ACVERSLAQL	PQBSIEDLS*	

The cp6642 nucleotide sequence <SEQ ID 370> is:

60	1	ATGGCTACAA	TCTCACCAT	ATCTTTAACT	GTAGATCATC	CCCTAGTAGA
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51  CACTAAAAA  AATTCCTGCA  GCAACTTTGA  TAAGATTTCAG  TCTCGAATTC
101  TATTGATTAC  TGCANCTTTT  GCTGCTCTAG  TTACTATAGG  GACCTTACTT
151  ATTTGGTTTGC  TTTTAAATAT  TCCCTGTATC  TATTTCCTCA  CAGGAATTTT
201  ATTTATTGCT  GTTGTCTTTA  GCAACTTTAT  CCATTATAAA  CGAGCAACCA
5   251  CCGTCTTAAA  ACCGGTGTCT  TGTGGCAAGC  ACAAGAAGAT  AAAACCAAAA
301  AGGGCTTCCA  CCAACCTACA  GTATTCTTCT  ATCTCTAATG  CAATCAATCG
351  TTTCTAAAGAA  AACTGGGAAC  ACCAACCCAA  GGACTCTACG  AATCTCCCCG
401  CACCCCTCTGC  ATTACTCACA  GATAACCCCT  ACGAGATATG  GAAAGCTAAA
451  CATTCACATGT  TTTCCCTAGT  ATCCCTCCTA  CCGGAGGGCA  ATCCAGAACA
10  501  TCTCTTAATT  TCAGCTTTCC  AAAATTAGG  AAGACTCTTG  TTAATTGAAG
551  AAACCTCGCA  AATTCGCGCT  ATATCCTCCT  ACGTAGATAC  CACTCCCTCC
601  CCAAAATCCT  TGCTCAATGA  GCAATTCAG  GAAACCGGG  TAGAATAAAA
651  TACAGAACTC  CCGTGGGAG  ATTCAAGGAG  ACGTTTATAC  TGGCAACCCG
701  ATTTCCGAGG  CCGCTCTTTC  CTCCACAAA  TACCAACAAC  TCTTGAAGCC
15  751  ATCTACCAAT  ACTACTATGC  ACTCTATGTC  ACTTATATCC  AGACTGCGAT
801  CAATACGAAC  ACCCAAATTA  TCCAAATCCC  TTTATACAGC  TTGAGGGAGC
851  ATCTCTATTC  TAGAGAAATG  CCCCCGCAAT  CAAGAAATGC  ACAATCTTTG
901  GCTATGATTA  CAGCAGTAAA  ATACATGGCC  GAGCTGCACC  CAGAATATCC
951  GCTAACTATT  GCTTGTGTG  AAGATCCCT  AGCCCAACTA  CCTCAAGAAA
20  1001  GTATTGAGGA  TCTCTCTTAG

```

The PSORT algorithm predicts inner membrane (0.5288).

The proteins were expressed in *E.coli* and purified as GST-fusion products. The recombinant proteins were used to immunise mice, whose sera were used in Western blots (Figures 181-185) and for FACS analysis.

25 These experiments show that cp6301, cp6558, cp6630, cp6633 and cp6642 are surface-exposed and immunoaccessible proteins, and that they are useful immunogens. These properties are not evident from their sequences alone.

### Example 186

The following *C.pneumoniae* protein (PID 4376389) was expressed <SEQ ID 371; cp6389>:

```

30  1  MSEVKPLFIK  NDSFDLATQR  FQNLIIMLQR  QABIYNEYEE  KNARVQNEIK
51  EQKDFVKRCI  EDFEARGLV  LKEELASLIR  DFHDKAKAST  SMLIBPCITG
101  FYSISHQEBQ  RQRQERLQKM  AERYRDKQV  LEAVQVQBQD  MISSRVVVDD
151  SYFEEKEBQ  KVDNRKKEQD *

```

The cp6389 nucleotide sequence <SEQ ID 372> is:

```

35  1  ATGTCAGAAG  TGAAGCCTTT  GTTTTAAAAG  AATGACTCTT  TTGATTTGGC
51  AACTCAGAGA  TTCCAGAATC  TAATTAACAT  GCTACAAGAG  CAAGCCGAGA
101  TATATTACGA  GTATTGAAGAA  AAGAATGCTA  GGGTTTCAGAA  TGAGATTAAAG
151  GAGCAAAAGG  ACTTTGTGAA  AAGATGCATA  GAGGACTTTG  AAGCCAGAGG
201  ACTGGGGGTG  CTAAAAGAAAG  AGCTTGCTAT  TTTGACGCGT  GATTTCCTATG
40  251  ATAAAAGCAA  AGCAGAGACT  TCTATGCTCA  TTGAATGTCC  TTGTATTGGT
301  TTTTATTATA  GTATTTCATCA  GGAGGAACAA  AGGCAAAAGC  AAGAAAGGCT
351  TCAAAAGATG  GGTGAGCGCT  ATAGGGACTG  TAAACAAGTC  TTGAGGCTG
401  TCCAGGTGGA  GCAAAAAGAT  ATGATACTTT  CTAGAGTCTG  TGTGATGAC
451  AGCTACTTTG  AAGAAAGAAA  AGAAGAACA  AAGGTGGATA  ACAAGAGAA
45  501  AGAACAGGAC  TAG

```

The PSORT algorithm predicts cytoplasm (0.3193).

The protein was expressed in *E.coli* and purified as a GST-fusion product (Figure 186A) and also in his-tagged form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 186B) and for FACS analysis.

These experiments show that cp6389 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 187

The following *C.pneumoniae* protein (PID 4376792) was expressed <SEQ ID 373; cp6792>:

```

5      1 VLQEHFFLSE DVITLAQQLL GHKLITHEBG LITSGYIVTF EAYRGPPDKA
      51 CHAYNYRKQTQ RNRAMYLKGG SAYLYRCYGM HELLNVVTFP EDIETHAVLIR
      101 AILFDQKQEL MIQRQRWDRK PPHILLTNGFP KVCQALQISL ENNRQRLNTP
      151 ALYISKEKIS GLTLTATARIG IDYAQEYRDV FWRFLLSFED SGKVLG*

```

The cp6792 nucleotide sequence <SEQ ID 374> is:

```

10      1 GTGCTACAAG AACATTTTTT TCTATCGGAA GATGTAATTA CACTAGCGCA
      51 ACAGCTTTTA GGACATAAAC TCATCACAAC ACATGAGGGT CTGATAACTT
      101 CAGGTACAT TGTAGAAACC GAAGCGTATC GTGCCCTGTA TGACAAAGCA
      151 TGCCACGCCT ACAACTACAG AAAAAGCTCAG AGGACACAGAG CGATGTACCT
      201 GAAAGAGGCG TCTGCTTACC TCTACCGTGT CTATGCGCATG CATCACCTAT
      251 TGAATGTTGT CACTGGACCT GAGGACATTC CCCATGCGGT CTTGATCCGG
      301 GCCATCCFTC CTGATCAAGG CAAAGAACTT ATGATCCAA CCGCCCAATG
      351 GAGAGATAAA CCCCCACACC TTCTCACCAA TGGACCCGGA AAAGTGTGCC
      401 AAGCTCTAGG AATCTCTTTG GAAACCAATA GGCAACGCTT AAATACCCCA
      451 GCTCTCTATA TCAGCAAGA AAAAAATCTCT GGAATCTTAA CAGCAACTGC
      501 CCGGATCCGG ATCGATTATG CTCAGAGSTA TCGTGTATGC CCATGGAGAT
      551 TTCTCTATTC CCGAGAAGAT TCGGGAAAAG TTTTATCTTA A

```

The PSORT algorithm predicts cytoplasm (0.180).

The protein was expressed in *E.coli* and purified as a his-tagged product (Figure 187A; lanes 2-4).

The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 187B) and for FACS analysis.

These experiments show that cp6792 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 188

The following *C.pneumoniae* protein (PID 4376868) was expressed <SEQ ID 375; ep6868>:

```

30      1 MVEIVLHNFQ RYLSKYLYRV FRFPCKKTF LSSHRVLARP SPFDVYCPGK
      51 TYDLQETIYEE LNAQLFQCAI RLQIGWFRKK ATTRKGSUVL GLPHENBQLI
      101 RIHRLSDRQE IPRPFMEYLV YHBMVHEVVP REXSLSGRSI FHGKKFKYEY
      151 QRPLLYDRAV AWEKANAYLI RGYKKRVGGG YGRA*

```

The cp6868 nucleotide sequence <SEQ ID 376> is:

```

35      1 ATGGTTGAAA CAGTACITCA TAAATTTCCAA CGTTATCTGA GCAGATATCT
      51 CTATAGGGTA TTTGCTTACC CATGTCGTAA AAAGACGTTT CTATCTTCGC
      101 ACAGGGTTCT TGCTCGTCTT TCAATCCGAG TAGACTACTG TCCGGGAAAG
      151 ATCTATGATT TGCAAGAGAT CTATGAGGAA TTGAATGCGC AGTTATTTCA
      201 AGGTGCACGT GGTTCACAGA TTGGTTGGTT CGGAAGGAAA GCTACCAAGA
      251 AAGGCAAGAG TGTTCCTCTG GGAATGTTTC ATGAAAATGA ACAATTAATT
      301 CGAATTCATC GTTCTTTAGA TCGGCAGGAA ATCCCAAGAT TTTTATGGA
      351 ATATCTTTGTG TATCATGAAA TGGTTCATAG TGTAGTCCCT AGAGAGTATT
      401 CTCTATCCGG GCGTTCGATT TTTTCATGGTA AAAAGTTTAA AGAATACGAA
      451 CAACGCTTCC CTTTGTATGA TCGTCTGTGT GCTTGGGAAA AGGCAACGCG
      501 TTTATTATTG CGAGGTGATA AAAAAAGAGT AGGTGAGGAA TATGCCAGGG
      551 CATAG

```

The PSORT algorithm predicts bacterial cytoplasm (0.325).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 188A; lanes 2-3). The recombinant protein was used to immunise mice, whose sera were used in a Western blot (Figure 188B) and for FACS analysis.

These experiments show that cp6868 is a surface-exposed and immunoaccessible protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 189

The following *C.pneumoniae* protein (PID 4376894) was expressed <SEQ ID 377; cp6894>:

```

1 MYKRCVLDKI LKGTVAGSLI LLVWSSDLE RDIKSTIKNV RDIQEDIRRI
51 SRVVKQCKTS QAIAPAQGVV LAHLVDRDEA FALLPDDPSY PNLISLDPYK
101 QQTLPPELLGT NPHPHGILRT AHVUKPENLS PFNGPDYVVG FYDLCLPSLA
151 SPHVGVKIEEF SPDLAVKIEE HLVEDGSGDK KPHIYLRPNV FWRPDPKAL
201 PKHVQLDEVF QRPHVPTAID IKFTYDAVMN PVVATMRAVA LRSCYEDVV9
251 VSVENDLKLVL VRNKHTVIN EKGKERRKVL YSAFSNPLSL QPLPRFVVOY
301 FANGEKIIED ENIDTYRTNS IWAQNFPMHW ANNYIVUSQGA YYPAGMDDK
351 IVFSRNPDMFY DPLAALIDKR FVYKPESTDS LQPDFTCKGI DISYLPNQR
401 DNFYSFMKSS AYNKQVARGG AVRETVSADR AVTYIGWNCF SLPTQSGQVR
451 CAMNMAIDRE RIIEQCLDGG GYTISGPPAS SSPSYNKLQLE GWHYSPEEAA
501 RLLEBEGNID TDGDIKREKV IDGVIVPFRF RLCYYVKSVT AHTIADYVAT
551 ACKKIGIECS LLGLMDALLS QAFDEKNPDA LLMGWLGLIP PEDPRALNHS
20 601 EGAMEKGSAN VVGPHNBEAD KIIDRLGYBY DLKERNRLVH RFHEIHEEBA
651 PYAFPLFSHHC SLLYKDYVKN IFVPTHRTDL IPEAQVETVN VTMVWLEKKE
701 DPCLSTS*
```

The cp6894 nucleotide sequence <SEQ ID 378> is:

```

1 ATGTATAAAA GATGTGTGCT AGATAAAATT TTAAGAGGGA TTGTCGCCGG
51 TTCTTTAATTT TTGTATATCT GGTCCTCAGA CCTACTTGAA AGAGACATTA
101 AGTCGATAAAA AGGTAACGTA AGAGATATTC AAGAAGACAT TCGTGAARCT
151 TCACGCGTAGT TGAACAACAA GCAGACATCA CAAGCTATCC CTGGCGCACC
201 TGGGGTGTATG CTGCTCTCTA AGCTCGTCAG AGACGAAGCT TTTGCTCTAC
251 TCTTTGGAGA TCTCTAGTTAT CTTAATTTAC TTTTCCCTAGA CCCCTATAAA
301 CAGCAGACTC TTCTTGAAC TCTAGGAAACA AATTTCACCC CTCATGGTAT
351 CCTACGCACT GCCCATGTGG GAAAACCCGA AAATCTGAGC CCTTTAATG
401 GCTTTGATTA TGTCTGGGGC TTTTACGATC TCTGTATCCC TAGTTAGCT
451 TCTCCGCCAG TAGGGAAATA CGAAGAATT TCTCCAGATC TCGCTGTGAA
501 AATAGAGAA CACTTTGTTG AAGATGCTTC TGGGGATAAA GAGTTCTACA
551 TCTATCTGAG CCGCAATGCT TTTTGGCGTC CTATAGATCC TAAAGCCCTT
601 CCAAAACACG TTCAGTTAGA CGAAGATATT CAACGTCTCT AATCTGTGAC
651 AGCTCATGAT ATTAAAGTTT TCTACGACGC TGTATGAAC CCTTAGTAG
701 CAACCATGCG AGCATGGCT CTGCGCTCTT GTTATGAAGA TGTGGTTCTT
751 GTCTCAGTAG AAAACGATTT AAAATTAGTA GTCAAGATGA AAGCACACAC
801 GGTAAATCAAT GAAGAAGGAA AGGAAGAGCG CAAGCTCTCT TACTCTGCAT
851 TTTCTAAATC CTTAAGCTTG CAGCCCTCC CTAGATTTGT ATATCAGTAT
901 TTTCTTAACG GGGAAAAAAT CATTGAAGAT GAGAAATATG ATACCTACCG
951 AACCAATPCC ATTGTGGCGC AAAACTTCAC TATGCAPTGG GCAACMACT
1001 ATATTGTAAAG TTGTGGAGCC TACTACTTTG CAGGGATGGA TGATAGRAAA
1051 ATCGGTGTTT CTAGAATAAC TGACTCTTAT GATCCCTCTG GCGCTCTTAT
1101 TGACAAGCGT TTGCTCTATT TTAAGGAAGG CACAGACTCC CTATTCCAAG
1151 ATTTTAAGAC AGGGAANAATA GACATCTCTT ACCTTCCACC CAACCAAGA
1201 GATAATTTCT ATAGTTTTAT GAAAAGCTCC GCTTATAACA AACAGGTAGC
1251 TAAGGAGGGA GCCGTCCGTG AAACAGTCTC AGCAGATCGA GCATATACGT
1301 ACATAGGATG GAATGTCTTT TCTATTATT TCCAAAGCCG ACAGGTGCGC
1351 TTGCTCTARGA ACATGGCAAT CGATAGAGAG AGATATATCG AACAGTGCTT
1401 GGAATGGCAA GGTCTACAGA TTAGTGGGCC TTTTGTCTCG AGTTCTCCCT
1451 CTATATAATAA ACAGATGGA GGGTGGCAAT ATTCCTCAGA AGAAGCAGCT
1501 CGTCTCTTGG AAGAAGAGGG ATGGAATAGT ACCGATGGCG ATGGAATCCG
1551 AGAAAAAGTT ATCGATGGTG TGATTGTCCC GTTCCCTTTC GSTTTATGCT
1601 ATTATGTAAA GAGTGTCACC GCTCTTACCA TGGCAGATTA GTAGTACTACT
1651 GCTTGTAAAG AAATGGGAAT CGAGTGTAGC CTCTTAGGAC TAGATATGGC
1701 CGATCTTTTCG CAGACTTTTG ATGAAAAGAA TTTCTATGCT CTTTTATGCG
1751 GATGGTGTAT AGGAATTCCT CCTAGAGATC CTAGGCGTTT ATGGAATCTT
```



```

1801 GAAGGGGCTA TGGAAAAGG TCTAGCGAAT GTTGTAGGTT TCCATATGA
1851 AGAAGCTGAT AAAATCATAG ACAGACTCAG CTACGAATAC GATCTGAAAG
1901 AACGTAATCG CCGTACCCAC CGTTTCCAAT AATTAATCA TGAAGGAAGCT
1951 CCTTATGCTT TCTTGTCTCT ACGACATGTT TCCTTACTTT ATAAGGATTA
2001 TGTAAAAAAT ATTTGCTGAC CTACACATAG AACAGATTTA ATTCCTGAAG
2051 CTCAGGATGA GACTGTCAAC GTAACATATG TATGGCTTGA GAAGAAGGAG
2101 GATCCGTGCT TAAGTACATC CTA

```

The PSORT algorithm predicts inner membrane (0.162).

The protein was expressed in *E.coli* and purified as a his-tag product (Figure 189A) and also in GST/his form. The recombinant proteins were used to immunise mice, whose sera were used in a Western blot (Figure 189B) and for FACS analysis.

These experiments show that cp6894 is a surface-exposed and immunoreactive protein, and that it is a useful immunogen. These properties are not evident from the sequence alone.

### Example 190

The following *C.pneumoniae* protein (PID 4377193) was identified in the 2D-PAGE experiment <SEQ ID 379; cp7193>:

```

1  MKRVIYKTF CGLTLLTSL SCSLDPRGYN LETKNSRDIN QESVILKRRR
51  ETPSLVKRLS RRRRLFPARR DQTQKDTLQV QANFKTYAEK ISEQDERDLS
101 FVSSAAEKS SLSLALSQGE TKDALYRIRE VHPALITLAL AENPALIEGM
151 KMKQGRDIW NLPFLQSLSEV FSQAWSQVI SEEDIAAFAS TLGLDSGTVA
201 SIVQGERWPE LWDIVIT*

```

A predicted leader peptide is underlined.

The cp7193 nucleotide sequence <SEQ ID 380> is:

```

1  ATGAAAAGAG TCATTATATA AACCATATTT TCGGGGTTAA CTTTACTTAC
51  AAGTTTGAGT AGTGTGTCCC TGGATCCTAA AGGATATAAC CTAGAGACAA
101 AAAACACGAG GGACTTAAAT CAAGAGTCTG TTAATATGAA GGAAACCGCT
151 GAAACACCTT CTCTGTATTA GAGACTCTCT CGTCGTTCTC GAAGACTCTT
201 CGCTCGACGT GATCAAACTC AGAAGGATAC GCTGCAAGTG CAAGCTAACT
251 TTAAGACCTA CGCAGAAAG ATTTCAAGAG AGGACGAAAG AGACCTTTCT
301 TCTGTTGTCT CGTCTGCTCG AGAAAAGTCT TCAATTTGCT TAGCTTTGTC
351 TCGAGGTGAA ATTAAGGATG CTTTGTACCG TATCCGAGAA GTCCACCTCT
401 TAGCTTTAAT AGAAGCTCTT GCTGAAAACC CTGCTTTGAT AGAAGGGATG
451 AAAAAGATGC AAGGCCGTGA TTGGAATTGG AATCTTTTCT TAACAACAT
501 AAGTGAAGTA TTTTCTCAAG CTTGCTCTCA AGGGGTTATC TCTGAAGAAG
551 ATATCGCGCG ATTTGCTTCC ACCTTAGGTT TGGACTCCGG GACCGTTGCG
601 TCCATTGTCC AAGGGGAAAG GTGGCCGAG CTGTGTGATA TAGTGATAC
651 TTA

```

The PSORT algorithm predicts periplasmic (0.925).

This shows that cp7193 is an immunoreactive protein in the EB and that it is a useful immunogen.

These properties are not evident from the protein's sequence alone.

It will be appreciated that the invention has been described by way of example only and that modifications may be made whilst remaining within the spirit and scope of the invention.

TABLE II – sequences of the primers used to amplify Cpn genes.

Orf ID	N-terminus final primer	C-terminus final primer
CP0014P	GGTTC CCG GGTCAATAG AAGTCTCTTTCCCA	GGT CTC GAG ATGAAGAGTTTFTGG
CP0015P	GGTCCCGGCTCAATG TCAGCTCTGTTTTCGA	GGT CTC GAG GAATGGTATTTGGTC
CP0016P	GGTCCCGGCTCAATG GCGAGCTCAACPAAG	GGT CTC GAG GTCCAGTAAAGTAGCA
CP0017P	GGT CCG GGTCAATG GGTATCAAGGGAAGT	GGT CTC GAG AAATCCGATCTCC
CP0019P	GGTCCCGGCTCAT ATGCAAGCTCTCAAGCATAG	GGT CTC GAG AAATCGGATATTACC
CP6280P	GGTTC CCG GGT GCTAGACATCGATTTCTTAAACC	GGT CTC GAG AAAAGCAAAATTCGCTC
CP6397P	GGTTC CCG GGTCAATATGTTTAACTGCTAAJAAATCTATT	GGT CTC GAG ATGAAGAAGAGTCCGTC
CP6456P	GGTTC CCG GGT CATATG TCACTCCGTAATAAACA	GGT CTC GAG CTGACATCTCCCTGT
CP6466P	GGTTC CCG GGT CAT ATG TGCAGGAGTCCAGT	GGT CTC GAG ATTTCTCTAGCATACG
CP6467P	GGTTC CCG GGT CAT ATG TGTTCCTCATCCAA	GGT CTC GAG TAGTTTCTATAAAGGAAGTCT
CP6468P	GGTTC CCG GGT CAT ATG TGTCTCCCTACTCTTC	GGT CTC GAG GGGGAAAGTAGGTATTTGA
CP6469P	GGTTC CCG GGT CAT ATG AGCTGCTCAAGAG	GGT CTC GAG ACTTAGATATCGATATTTTGA
CP6552P	GGTTC CCG GGT CAT ATG TGCCATAGGAAGCA	GGT CTC GAG ACCATGTCTTGATCAT
CP6567P	GGTTC CCG GGT CAT ATG AGCTCACGATCCCC	GGT CTC GAG AGAAGCCGTAGAGG
CP6576P	GGTTC CCG GGT CAT ATG ACTGAAGAGTTTAAAGAG	GGT CTC GAG GAA CTGCGCCCTAAG
CP6727P	GGTTC CCG GGT CATATGCTAGATCCACTAATGCC	GGT CTC GAG GAAGAATACAGGATCC
CP6728P	GGTTC CCG GGT CAT ATGCGAGATGCTCTTTATC	GGT CTC GAG GAATGATATCTTAGCC
CP6731P	GGTTC CCG GGT CATATGCGTGTGTGTGAATCAAT	GGTCT CAT GGC GGC GCG GAATCGGAACCTACCTCC
CP6736P	GGTTC CCG GGT GCT AGCGTAGAAGTTATCATGCGCTT	GGTCT CAT GGC GGC GCG AATCGGTAAATTCGCTC
CP6737P	GGTCT GGA TCC CAT ATG GAGCATAGACTGAGGAG	GGT CTC GAG AAATGTGAGATTAGTCC
CP6751P	GGTTC CCG GGT GCT AGC AATGAAGTCTCCAACT	GGT CTC GAG AAATCTCAATCTACTGCG
CP6752P	GGTGA ATT CAA ATGTGTGGGATGACTCTCT	GGT CTC GAG GAATTTAGAGTATCTCTG
CP6753P	GGTTC CCG GGT GCT AGCATCTCCCTACTCTCATAG	GGT CTC GAG AAATCTTAAGGTCGTCT
CP6767P	GGTTC CCG GGT CAT ATG ATAAACAATAAGGCGT	GGT CTC GAG TCTGTAGAGCACTTCAGA
CP6829P	GGTTC CCG GGT CAT ATG AAGCGAGTGCCTCTT	GGTCT CAT GGC GGC GCG GAATCTAAGGGAGAGC
CP6850P	GGTTC CCG GGT CAT ATG AGTCCCGGCTGCTGT	GGTCT CAT GGC GGC GCG GAACTCAACACCGGATCC
CP6852P	GGTTC CCG GGT CAT ATG CATAAAGTAAGTATTTTCATT	GGT CTC GAG TAACTAGAAAGAGCTCC
CP6849P	GGTTC CCG GGT CAT ATG TCACTCAATCTACATCCC	GGT CTC GAG AAACCGAGCATTTTATC
CP6845P	GGTTC CCG GGT GCT AGC AGCGGGGATATAGAG	GGT CTC GAG ATACAGCTGTGATTTTTC
CP6850P	GGTTC CCG GGT CAT ATG TCCCGCATTTGTAGT	GGT CTC GAG CTGTGTCATCTGCC
CP6854P	GGTTC CCG GGT GCT AGC TCAATAGCTATTGCCAG	GGT CTC GAG TTATCGAAATGCTCTTG
CP6879P	GGTTC CCG GGT CAT ATG GCAACACTGCTCA	GGTCT CAT GGC GGC GCG TCTCTAAATATKCTCTGC
CP6884P	GGTTC CCG GGT CAT ATG TATAAAGATGTGTGCTAGA	GGT CTC GAG GATATCTGTATACGAG
CP6900P	GGTTC CCG GGT CAT ATG AAGATLAAATTTCTCTGAG	GGT AAG CTT GGGAGAGCATACCC
CP6852P	GGTTC CCG GGT CAT ATG CTCTCGATCAATATNTAG	GGT CTC GAG TCGAATTTCTTTTATG
CP7034P	GGTTC CCG GGT CAT ATG AAAAAAGAGGTATATCAAG	GGT AAG CTT AAACGCTGAATATAC
CP7050P	GGTTC CCG GGT CAT ATG GTAGGCTTTCCCT	GGT CTC GAG GGTGTCAGATCTTA
CP7081P	GGTTC CCG GGT CAT ATG GAGAAATVAGAGTGTGTGT	GGT CTC GAG TAGTGTCTCTTTATCGGT
CP7170P	GGTTC CCG GGT CAT ATG CTAGGGGCTGGAACC	GGT AAG CTT AAATCGAGACTCGAG
CP7228P	GGTTC CCG GGT CAT ATG ACTGCTGTCTTATCTCTAC	GGT CTC GAG ATCTGAAGCGGAG
CP7249P	GGTTC CCG GGT CAT ATG AGCCCATCCCTCAT	GGT CTC GAG ATCAGGTGCTGAGACTT
CP7250P	GGTTC CCG GGT CAT ATG AATCTTCAACAGGCTCT	GGT CTC GAG ATTTTCTCAAGAGACTCTC
CP0018P	GTGCGT CATATG GCAACCACTCCACAA	ACTCGTA GCGGCCG TAATGAGTCCCGAC
CP6270P	GTGCGT CATATG AATTTATAGAGCTGTCT	ACTCGTA GCGGCCG AAATTTGATTTTCTATCC
CP6755P	GTGCGT CATATG GCAGCAAGTTGTATAT	ACTCGTA GCGGCCG TGCGTGAAGATGATC
CP6959P	GTGCGT CATATG TTGCGTGTAGGGAAC	ACTCGTA GCGGCCG GAATCTCACTGACCAAGA
CP7053P	GTGCGT CATATG GTTAACTCTATTGTGTCCA	ACTCGTA GCGGCCG TTGGAGATTAACCGAATATA
CP7287P	GTGCGT CATATG TTACACAGCTCAGAACTAGA	ACTCGTA GCGGCCG GAAATTAATACGATACCA
CP0010P	GTGCGT CATATG GCAACCTGGAARAATA	GGT CTGAG GAATGTGCACTTACCC
CP0408P	GTGCGT GCTAGC ATTTTTATAGCAAACTCTAT	GGT CTGAG AAATGTGCACTTACCTC
CP6272P	GTGCGT CATATG TTGACTCATCAAGAGCT	GGT CTGAG GAAGGAGTGTTTTATGGT
CP6273P	GTGCGT CATATG ACATATCTGHAAGCTC	ACTCGTA GCGGCCG CTCCAAATTTTATG
CP6382P	GTGCGT CATATG CCCTTGATATTAATTAATAGA	GGT CTGAG TGTGTTCCAAATCA
CP6372P	GTGCGT CATATG AAACAGCACTATCTCTAATA	GGT CTGAG TTTCTGTCTGATTTCT
CP6390P	GTGCGT CATATG CGAAGGTCCTTAAAG	ACTCGTA GCGGCCG TCTCTGAGCAAGCTT
CP6402P	GTGCGT CATATG AATTTGCGGATCTGCTTT	GGT CTGAG GAAGGATTTGCGGT
CP6446P	GTGCGT CATATG TGTAACTAAAGGCCCTCTT	GGT CTGAG GGGTCAGAGAGGAC
CP6520P	GTGCGT GCTAGC AAACATACCTATCATTTTCT	GGT CTGAG CAGAAAGGCTTTTCTT
CP6577P	GTGCGT CATATG AATTAGGCTATGTTAATTA	GGT CTGAG GTTTGTGTTTGAAGA
CP6602P	GTGCGT CATATG GCAGCATCAGAGGACA	GGT CTGAG TGACCAAGATAGGTTTATG

CP6607P	GTGCGT	CATATG	CTCGTGTGTGACACTT	GCCT	CTCGAG	CGCTGCTCTTCTGCTC
CP6615P	GTGCGT	CATATG	TGCTCTCAAAJAAAGCAAA	GCCT	CTCGAG	TGAAGAGCGCCATC
CP6624P	GTGCGT	CATATG	GATGCGAAATGGGA	GCCT	CTCGAG	TCTTTGACATTCAGAGC
CP6627P	GTGCGT	CATATG	ATTCCTACCACTGTAAATG	GCCT	CTCGAG	GTCACTAAATCTCTTATATA
CP6670P	GTGCGT	CATATG	TGCACTCACTTAGGCT	GCCT	CTCGAG	CGAGTAGTTAGACAAAC
CP6717P	GTGCGT	GCTAGC	AAGACAACGTAGCTTCA	ACTCGCTA	CGGCGCGC	GGCTGCTGATATAGGT
CP6784P	GTGCGT	GCTAGC	AAATCAAGATGTTCTATAGATA	GCCT	CTCGAG	TCCAAACAACCCCTCT
CP6802P	GTGCGT	CATATG	TGCGTAACTATATTAATTCCTT	GCCT	CTCGAG	CACTCGGACTGTGTG
CP6847P	GTGCGT	CATATG	TGCGATCTTTACAGAG	GCCT	CTCGAG	TCTTCTCACACTGTTATATAAA
CP6884P	GTGCGT	CATATG	AATCACTGCTCTTCT	GCCT	CTCGAG	AGAGAGGTATTTGTATCC
CP6888P	GTGCGT	CATATG	TGTCTACTATTATCTATCTCTAC	GCCT	CTCGAG	TTCAGAAATGGCT
CP6890P	GTGCGT	CATATG	TCCCCACAGACGACAA	GCCT	CTCGAG	TCTGTGACAGCTTAGC
CP6890P	GTGCGT	CATATG	TGTGACGTACGCTCTA	ACTCGCTA	CGGCGCGC	TTCACCTTGATTCCT
CP6898P	GTGCGT	CATATG	TGCGATGCGAAJAC	ACTCGCTA	CGGCGCGC	GGAAGTGTCTAGATATT
CP6898P	GTGCGT	CATATG	TGCTGTGTCTACTCTAT	ACTCGCTA	CGGCGCGC	AAAAGGTCTAGATATACCT
CP7005P	GTGCGT	CATATG	AAJAACTGTGATATTGAACA	GCCT	CTCGAG	CTAGGCTCTATTTCTATAT
CP7072P	GTGCGT	CATATG	CCCACTTATGSGAAA	GCCT	CTCGAG	GTTAGCGAAGGTGTTG
CP7101P	GTGCGT	CATATG	TATTCCTGTGTACAGCAA	GCCT	CTCGAG	GAAJAAATCTTAGGGAG
CP7102P	GTGCGT	CATATG	GCCTGTAAAGCAAT	GCCT	CTCGAG	TGAJAAATGAAGATGTG
CP7105P	GTGCGT	GCTAGC	AGTCTATATCAJAAATGCTG	GCCT	CTCGAG	ATCTTTGATTTGGTACT
CP7106P	GTGCGT	CATATG	AAAGAATTGGGAACTCT	GCCT	CTCGAG	GAATCTCAAGGCATATCTA
CP7107P	GTGCGT	GCTAGC	AGTATAGTCAJAAATCTGCA	GCCT	CTCGAG	GAGCTAGATTTATAGTACTTT
CP7108P	GTGCGT	GCTAGC	GGGCGCTTTCCA	ACTCGCTA	CGGCGCGC	TTATATATATGGAACATAGG
CP7109P	GTGCGT	CATATG	GAACATTTATGATAATG	ACTCGCTA	CGGCGCGC	ATCATCAGGTAGAAJAG
CP7110P	GTGCGT	CATATG	GATTAATGCTATGATAATACA	GCCT	CTCGAG	TTCGTATGAGCTCCA
CP7127P	GTGCGT	CATATG	GTGGCTTTAAGCATAGC	ACTCGCTA	CGGCGCGC	CGAGCTATCTGATTC
CP7130P	GTGCGT	CATATG	TTCATATCTGGGAG	GCCT	CTCGAG	CTTCTATTTGGAATTTG
CP7140P	GTGCGT	CATATG	ACAGCCGGAACAGCT	GCCT	CTCGAG	AGCACCTCTAATTTGATG
CP7182P	GTGCGT	CATATG	GAATATGTTCTATATGATC	GCCT	CTCGAG	GCTACTAACTGAATCGA
CP6262P	GTGCGT	CATATG	ATCCCTGTGATTAAGTTCA	ACTCGCTA	CGGCGCGC	TTCATCGGAGCTTGA
CP6268P	GTGCGT	CATATG	TACCAGGAGAACTAAGAT	ACTCGCTA	CGGCGCGC	GATTTCTTCTTCACTC
CP6298P	GTGCGT	CATATG	GAGGAGGTGTCTGAGAT	ACTCGCTA	CGGCGCGC	ATGTTCTTTTACTCTTCT
CP6419P	GTGCGT	CATATG	GCTCAAGTCTCGTGT	GCCT	CTCGAG	AAGTGTTCGTGGAGAT
CP6601P	GTGCGT	CATATG	AAATAGCTACTCAATTCGT	GCCT	CTCGAG	GAAATCTGGAATTCCTCT
CP6639P	GTGCGT	CATATG	TTJAAATCAAGCAATCA	GCCT	CTCGAG	AGGAATCAAAACCTCATCT
CP6664P	GTGCGT	GCTAGC	GTTTATTTTCACGCTCA	ACTCGCTA	CGGCGCGC	CTTAGAAGACATATTTCTTAAGTA
CP6896P	GTGCGT	CATATG	TGCTGTGATATGCG	GCCT	CTCGAG	ATTCATCTTCTGATAAGAT
CP6757P	GTGCGT	CATATG	GCAGTTGTGTGGCT	ACTCGCTA	CGGCGCGC	CTGTCCCTCTGGAGC
CP6790P	GTGCGT	GCTAGC	AGTGAJACAJAAJAAATCA	ACTCGCTA	CGGCGCGC	CTTATCTCTCTTATCAATA
CP6814P	GTGCGT	CATATG	CATGACGCACTCTTAAG	GCCT	CTCGAG	TACAGTGTGCGGA
CP6834P	GTGCGT	CATATG	GTATAGGGAACCTATATCG	GCCT	CTCGAG	TACATTTGTATATTTCTAG
CP6878P	GTGCGT	CATATG	AACCTCCGTGATCC	GCCT	CTCGAG	GCTAGCGGCTCTTTC
CP6899P	GTGCGT	CATATG	CAGAAAGCACTCTGCT	ACTCGCTA	CGGCGCGC	TCTCTTTAGAGAAJAG
CP6906P	GTGCGT	CATATG	TCTCTTTAGGAAATGG	GCCT	CTCGAG	CACTGTGCAAGTGA
CP7015P	GTGCGT	CATATG	GCAGTACGATTAATTTGTG	GCCT	CTCGAG	TTTATTTGATCTTTATATATTC
CP7035P	GTGCGT	GCTAGC	AGCAGAAJAGCAATGA	GCCT	CTCGAG	ATTTTGAAGTCTCTGCA
CP7073P	GTGCGT	CATATG	ATTACCAATACTACGTG	GCCT	CTCGAG	TATCTCTGACATATAGAG
CP7085P	GTGCGT	GCTAGC	TGTATTTTCCCTTACGTG	ACTCGCTA	CGGCGCGC	GGATTCTGCACTACTG
CP7092P	GTGCGT	CATATG	TCTCTCTTCTCTAATAAAA	GCCT	CTCGAG	GGATTCTATCTAGACCA
CP7093P	GTGCGT	CATATG	AAATACCGCTTCAAG	GCCT	CTCGAG	ATTCCTGTAGGCTACTG
CP7094P	GTGCGT	CATATG	GTACACTTCTCTATAACC	GCCT	CTCGAG	TAAATTTGTATTTGGGTAT
CP7132P	GTGCGT	CATATG	TGTATTAAGGAGCTTAGGA	GCCT	CTCGAG	TTTCCCAACCCCA
CP7133P	GTGCGT	CATATG	GCTGCGAATGCTC	GCCT	CTCGAG	TAAATTAATCTCTTTGAAG
CP7177P	GTGCGT	CATATG	CTTACTCAAGTTAAJACAGA	GCCT	CTCGAG	AAGTTTATATTTTACGACATT
CP7184P	GTGCGT	GCTAGC	CATATAGGATTTTGCCA	GCCT	CTCGAG	GTACTTAGCAAGCAT
CP7206P	GTGCGT	GCTAGC	AGAGAGCTATACACCTA	GCCT	CTCGAG	CACACCGAGGAJAC
CP7222P	GTGCGT	CATATG	GTAGTTTCAAGAGAAAAGTC	GCCT	CTCGAG	ACGTATGTGCGACAT
CP7223P	GTGCGT	CATATG	GAAGTATTAAGACGCTCT	GCCT	CTCGAG	CGAGAAJAGCTTCC
CP7224P	GTGCGT	CATATG	ATGAGAAJAAATCTGAAA	ACTCGCTA	CGGCGCGC	TAGCATCTACAAATGA
CP7225P	GTGCGT	CATATG	CATATTTTGTGTGATCT	GCCT	CTCGAG	TCTTTTAACTAAJCTTTGTCTT
CP7303P	GTGCGT	CATATG	CTTGCTCTATTGTTTGAATCC	GCCT	CTCGAG	AAATATCAAGAACTCCG
CP7304P	GTGCGT	GCTAGC	GAGTTTATAGATTTTTC	GCCT	CTCGAG	TTTTTGAATCTTAAJAG
CP7305P	GTGCGT	CATATG	GAGTTTATAGATTTTTC	GCCT	CTCGAG	ACTCTCTGAGAGGGGA
CP7307P	GTGCGT	CATATG	CTTAACTATGCTAAJAG	ACTCGCTA	CGGCGCGC	CTCTTTTATTTAGGAAGCT

CP7342P	GTGCGT CATATG AAAAAAAATCTATTCTCACT	ACTCGCTA GCGGCCG CACACTCTGTCTCTG
CP7347P	GTGCGT CATATG TTCTCTAGGATTTGACTAA	GCCT CTCGAG CGAAGCAGAGTCGT
CP7353P	GTGCGT CATATG AATATGCTCTTCTCTCT	GCCT CTCGAG GGGCGTAGAGTTGA
CP7183P	GTGCGT CATATG TGTTCCTGGATCCT	ACTCGCTA GCGGCCG AGTATCACTATATCCACAAG
CP7248P	GTGCGT GCTAGC CTTGAACATTTCTAACAGAT	GCCT CTCGAG ACGTAGTTTALGAGCAGACT
CP7261P	GTGCGT CATATG TGTCTATCTGCTTACATAG	GCCT CTCGAG TTITGATGCTTCTTTCA
CP7280P	GTGCGT CATATG GACCGAAGAAATTGAAAA	GCCT CTCGAG AGAGGCTCTCTGAGTGC
CP7302P	GTGCGT CATATG AATTTCCATCTGATGTAGT	GCCT CTCGAG GAACAGTTCGATTTGTG
CP7306P	GTGCGT CATATG CTTCTCTTATCAGGGCA	ACTCGCTA GCGGCCG TCTTTCAGGTTTCAGG
CP7367P	GTGCGT GCTAGC CPTTATGCCAGGTC	GCCT CTCGAG TTCGTGCTTTTGGTG
CP7408P	GTGCGT CATATG TTGAATATCCAGNAAA	GCCT CTCGAG ATTCAATTTCCGAGAG
CP1409P	GTGCGT CATATG AGACGTTATCTTTTACATGCT	GCCT CTCGAG CCGTTTGTCTTTTACATAG
CP6733P	GTGCGT ACTAGT TGTACCTACAGTCACATAG	GCCT CTCGAG GAATCGAGGTTTGGTA
CP6728P	GTGCGT ACTAGT AAGTCCCTCTGTCTCTTGG	GCCT CTCGAG GAACACAACTAGAGCCC

TABLE III – Proteins with best results in FACS analysis

cp number	Molecular Weight (kDa)		Fusion type
	Theoretical	Western Blot	
6260	97.5	94; 70	GST
6270	87.5	-	GST
6272	78.0	90	GST
6273	58.6	74; 64; 50	GST
6296	31.1	-	GST
6390	88.9	102	GST
6456	42.5	89; 67,45	GST
6466	57.5	59; 56	His
6467	59.0	67	GST
6552	28.4	50; 27	GST
6576	86.0	79; 70; 62; 45	GST
6577	17.3	12	GST
6602	43.4	53; 42; 34	GST
6664	54.5	104; 45	GST
6696	47.9	95; 53	GST
6727	130.0-142.9	123; 61; 39	His
6729	94.8	multiple bands	GST
6731	95.5	97	GST
6733	97.1	104	His
6736	100.1	98; 93; 66; 60	GST
6737	101.2	multiple bands	GST
6751	100.2	95; 71	GST
6752	102.1	97; 48	His
6767	29.1	28	GST
6784	32.9	35	GST
6790	71.3	multiple bands	His
6802	29.7	-	GST
6814	29.6	28	GST

6830	177.4	174; 91; 13	GST
6849	57.3	multiple bands	GST
6850	7.4-9.4	61; 14; 8	GST
6854	42.2	-	GST
6878	40.4	-	GST
6900	28.0	-	GST
6960	25.6	75; 35	GST
6968	34.6	83; 53; 35	GST
6998	39.3	multiple bands	GST
7033	68.2	multiple bands	GST
7101	113	105	GST
7102	63.4	-	GST
7105	29.2	30	GST
7106	39.5	72; 46	GST
7107	71.4	67; 31	His
7108	35.9	35	GST
7111	46.1	51	GST
7132	17.9	57; 47; 17	His
7140	36.2-29.8	50; 38; 34	GST
7170	34.4	77; 33	GST
7224	39.4	40	GST
7287	167.3	180	GST
7306	50.1	50	GST

TABLE IV – FACS-positive proteins not found in *C.trachomatis*

cp7105	cp6390
cp7106	cp6784
cp7107	cp6296
cp7108	

TABLE V – Proteins identified by MALDI-TOF following 2D electrophoresis

cp6270	cp6733	cp6900
cp6552	cp6736	cp6960
cp6576	cp6737	cp6998
cp6577	cp6752	cp7033
cp6602	cp6767	cp7108
cp6664	cp6784	cp7111
cp6727	cp6790	cp7170
cp6728	cp6830	cp7287
cp6729	cp6849	cp7306

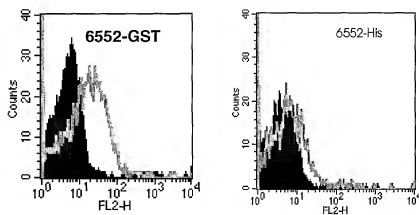
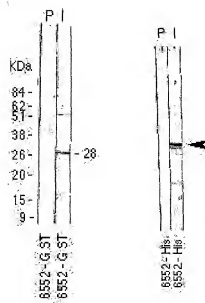
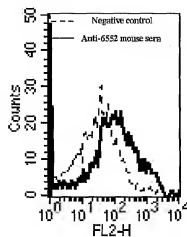
## CLAIMS

1. A protein comprising an amino acid sequence selected from the group consisting of SEQ IDs 97,  
1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53,  
55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 99, 101, 103, 105,  
5 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143,  
145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181,  
183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219,  
221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257,  
259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295,  
10 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333,  
335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371,  
373, 375, & 377.
2. A protein having 50% or greater sequence identity to a protein according to claim 1.
3. A protein comprising a fragment of an amino acid sequence selected from the group consisting of  
15 SEQ IDs 97, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47,  
49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 99,  
101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137,  
139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175,  
177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213,  
20 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251,  
253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289,  
291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327,  
329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365,  
367, 369, 371, 373, 375, & 377.
- 25 4. A nucleic acid molecule which encodes a protein according to any one of claims 1 to 3.
5. A nucleic acid molecule according to claim 4, comprising a nucleotide sequence selected from  
the group consisting of SEQ IDs 98, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34,  
36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86,  
88, 90, 92, 94, 96, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128,  
30 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166,  
168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204,  
206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242,  
244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280,  
282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318,

320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, & 378.

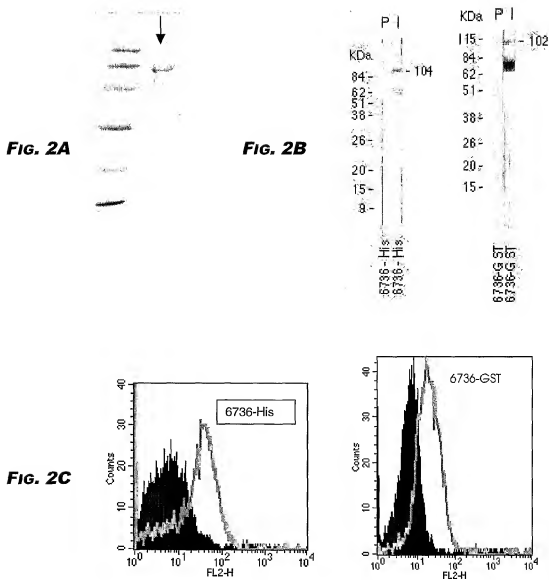
6. A nucleic acid molecule comprising a fragment of a nucleotide sequence selected from the group consisting of SEQ IDs 98, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40,  
5 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248,  
10 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, & 378.
7. A nucleic acid molecule comprising a nucleotide sequence complementary to a nucleic acid molecule according to any one of claims 4 to 6.
8. A nucleic acid molecule comprising a nucleotide sequences having 50% or greater sequence identity to a nucleic acid molecule according to any one of claims 4 to 7.
9. A nucleic acid molecule which can hybridise to a nucleic acid molecule according to any one of claims 4 to 8 under high stringency conditions.
- 20 10. A composition comprising a protein or a nucleic acid molecule according to any preceding claim.
11. A composition according to claim 10 being a vaccine composition.
12. A composition according to claim 10 or claim 11 for use as a pharmaceutical.
13. The use of a composition according to claim 10 in the manufacture of a medicament for the treatment or prevention of infection due to *Chlamydia* bacteria, particularly *Chlamydia pneumoniae*.
- 25

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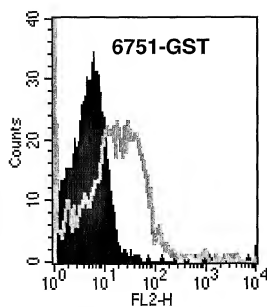
**FIGURE 1****FIG. 1A****FIG. 1B****FIG. 1C**



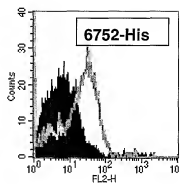
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**FIGURE 2**

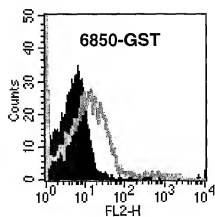
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**FIGURE 3****FIG. 3A****FIG. 3B****FIG. 3C**

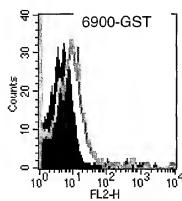
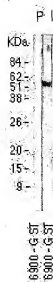
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**FIGURE 4****FIG. 4A****FIG. 4B****FIG. 4C**

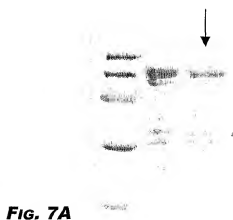
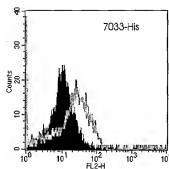
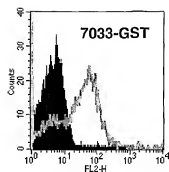
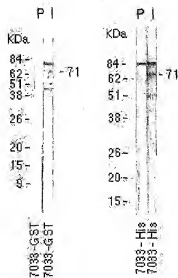
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**FIGURE 5****FIG. 5A****FIG. 5B****FIG. 5C**

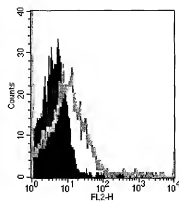
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**FIGURE 6****FIG. 6A****FIG. 6B****FIG. 6C**

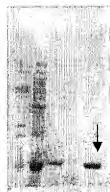
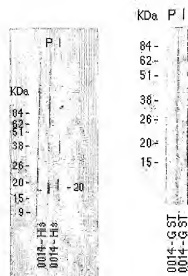
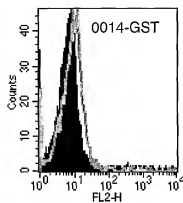
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**FIGURE 7****FIG. 7A****FIG. 7B****FIG. 7C**

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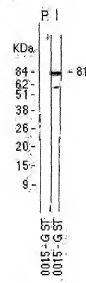
**FIGURE 8****FIG. 8A****FIG. 8B****FIG. 8C**

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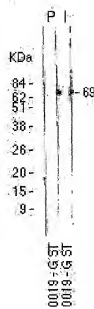
**FIGURE 9****FIG. 9A****FIG. 9B****FIG. 9C**



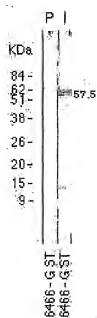
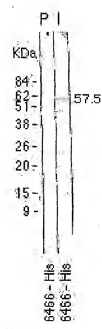
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**FIGURE 10****FIG. 10A****FIG. 10B**

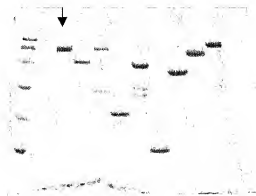
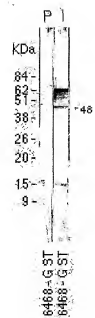
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**FIGURE 11****FIG. 11A****FIG. 11B****FIG. 11C**

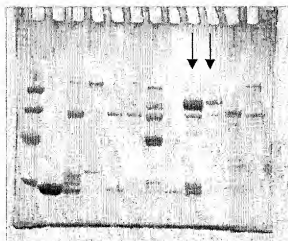
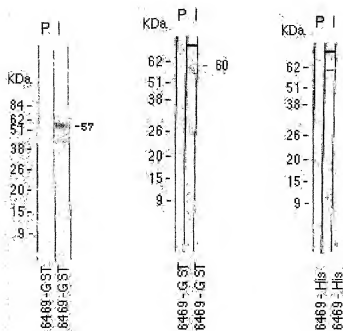
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**FIGURE 12****FIG. 12A****FIG. 12B****FIG. 12C**

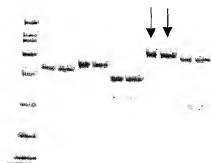
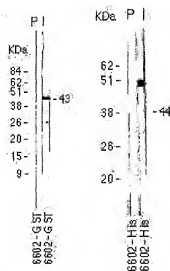
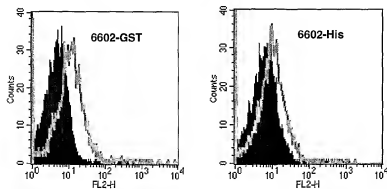
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**FIGURE 13****FIG. 13A****FIG. 13B**

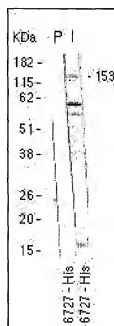
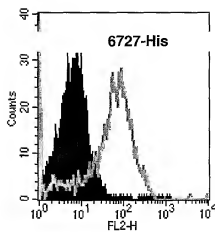
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**FIGURE 14****FIG. 14A****FIG. 14B**

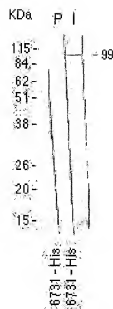
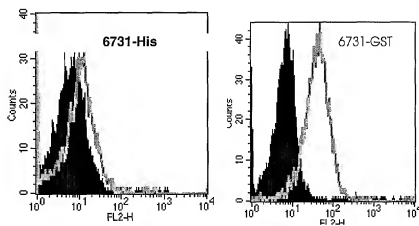
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**FIGURE 15****FIG. 15A****FIG. 15B****FIG. 15C**

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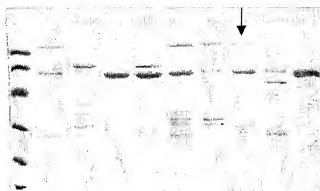
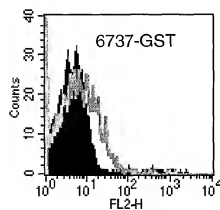
**FIGURE 16****FIG. 16A****FIG. 16B****FIG. 16C**

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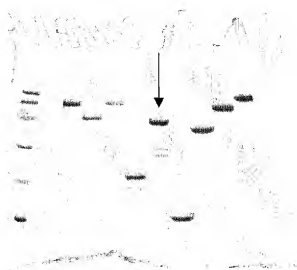
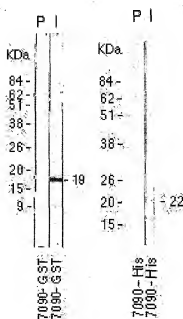
**FIGURE 17****FIG. 17A****FIG. 17B****FIG. 17C**



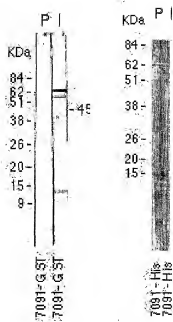
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**FIGURE 18****FIG. 18A****FIG. 18B****FIG. 18C**

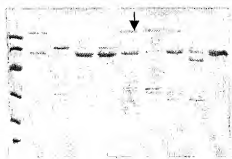
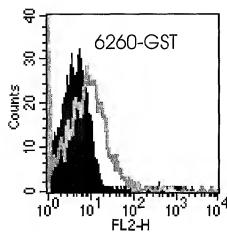
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**FIGURE 19****FIG. 19A****FIG. 19B**

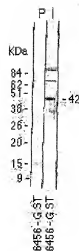
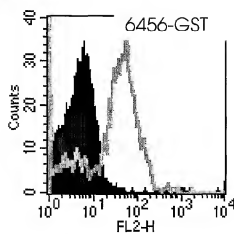
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**FIGURE 20****FIG. 20A****FIG. 20B**

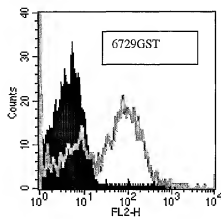
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**FIGURE 21****FIG.  
21A****FIG.  
21B****FIG.  
21C**

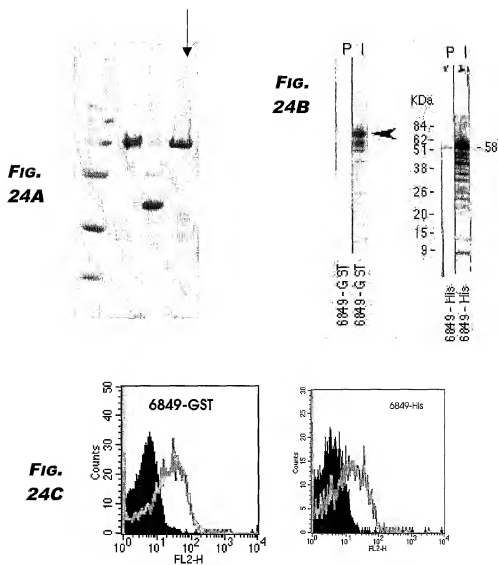
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**FIGURE 22****FIG.  
22A****FIG.  
22B****FIG.  
22C**

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**FIGURE 23****FIG.  
23A****FIG.  
23B****FIG.  
23C**

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**FIGURE 24**

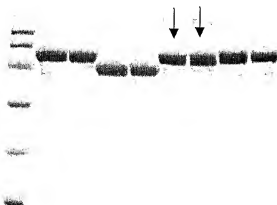
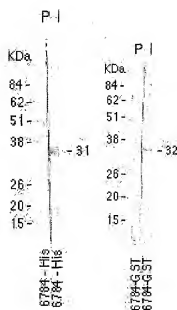
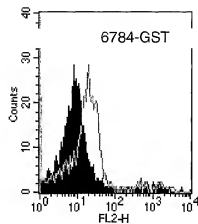




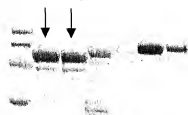
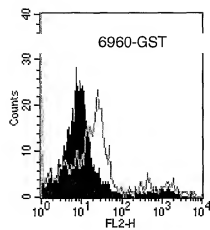
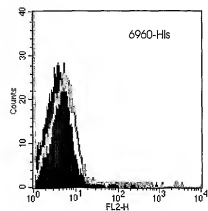
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**FIGURE 26****Fig. 26A****Fig. 26B**

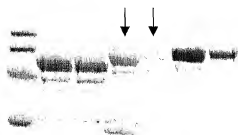
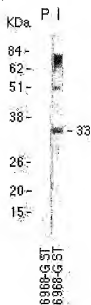
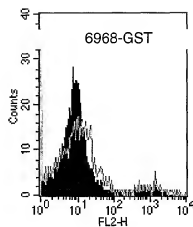
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**FIGURE 27****FIG. 27A****FIG. 27B****FIG. 27C**

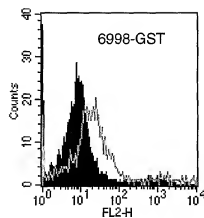
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**FIGURE 28****FIG. 28A****FIG. 28B****FIG. 28C**

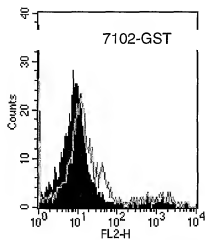
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**FIGURE 29****FIG. 29A****FIG. 29B****FIG. 29C**

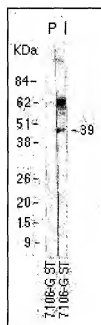
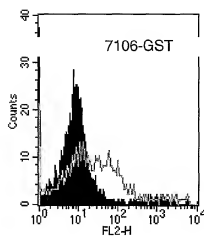
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**FIGURE 30****Fig. 30A****Fig. 30B****Fig. 30C**

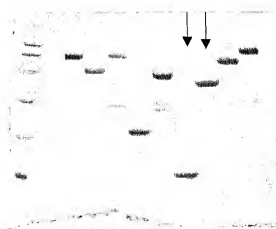
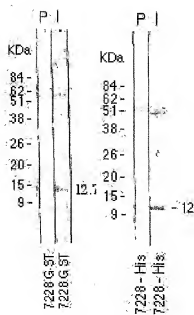
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**FIGURE 31****Fig. 31A****Fig. 31B**

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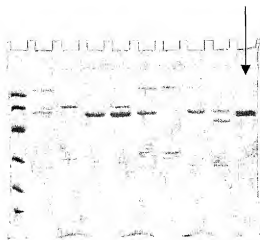
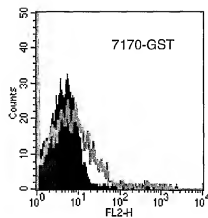
**FIGURE 32****FIG. 32A****FIG. 32B****FIG. 32C**

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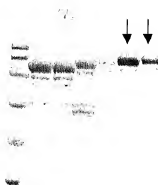
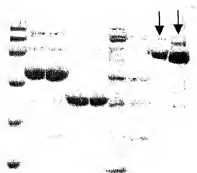
**FIGURE 33****Fig. 33A****Fig. 33B**



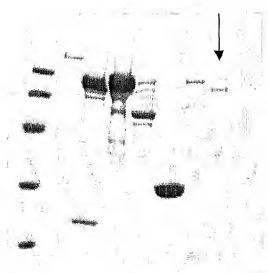
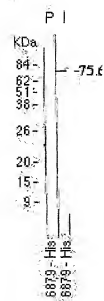
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**FIGURE 34****FIG. 34A****FIG. 34B****FIG. 34C**

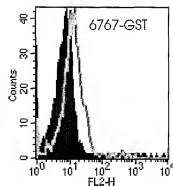
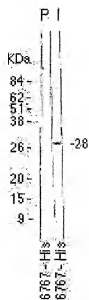
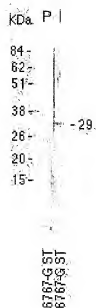
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**FIGURE 35****FIG. 35A****FIG. 35B****FIG. 35C**

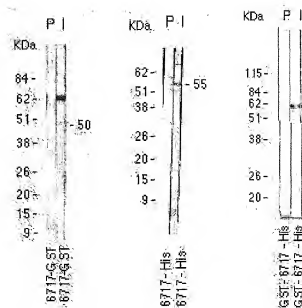
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**FIGURE 36****FIG. 36A****FIG. 36B**

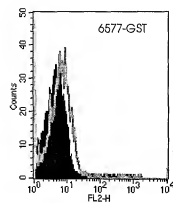
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**FIGURE 37****FIG. 37A****FIG. 37C****Fig. 37B****Fig. 37D**

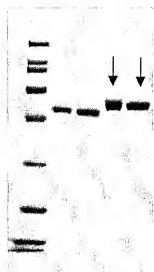
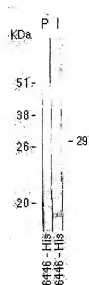
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**FIGURE 38****FIG. 38A****FIG. 38B**

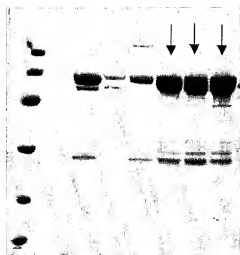
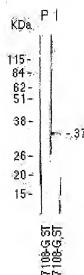
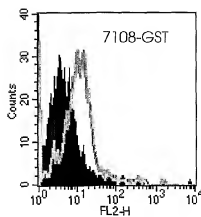
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**FIGURE 39****FIG. 39A****FIG. 39B****FIG. 39C****FIG. 39D**

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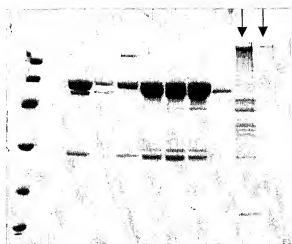
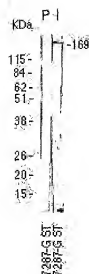
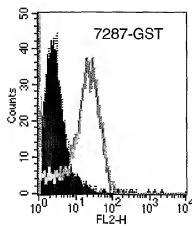
**FIGURE 40****FIG. 40A****FIG. 40B**

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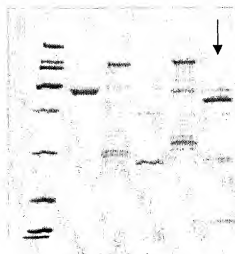
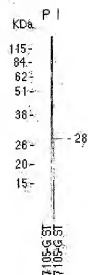
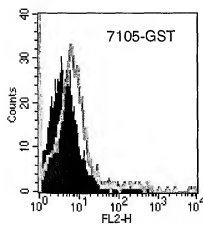
**FIGURE 41****Fig. 41A****Fig. 41B****Fig. 41C**



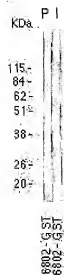
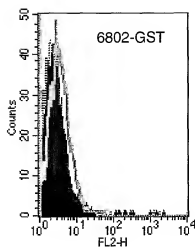
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**FIGURE 42****FIG. 42A****FIG. 42B****FIG. 42C**

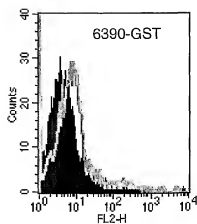
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**FIGURE 43****FIG. 43A****FIG. 43B****FIG. 43C**

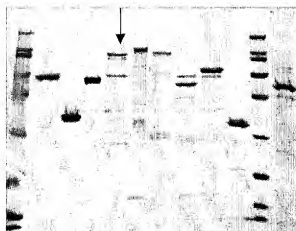
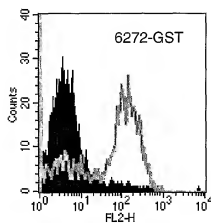
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**FIGURE 44****FIG. 44A****FIG. 44B****FIG. 44C**

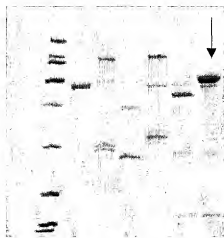
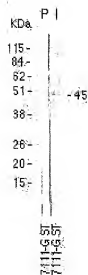
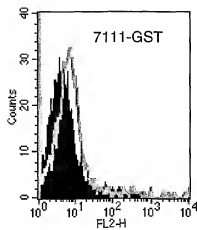
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**FIGURE 45****FIG. 45A****FIG. 45B****FIG. 45C**

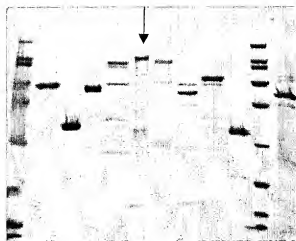
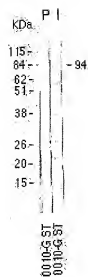
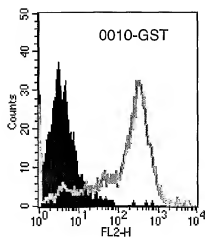
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**FIGURE 46****FIG. 46A****FIG. 46B**

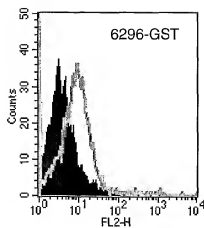
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**FIGURE 47****FIG. 47A****FIG. 47B****FIG. 47C**

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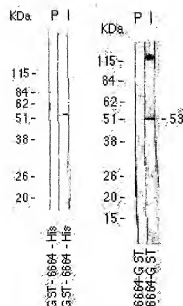
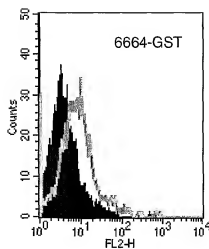
**FIGURE 48****FIG. 48A****FIG. 48B****FIG. 48C**

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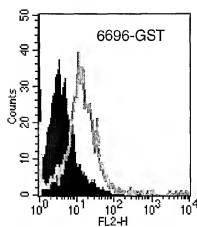
**FIGURE 49****Fig. 49A****Fig. 49B****Fig. 49C**



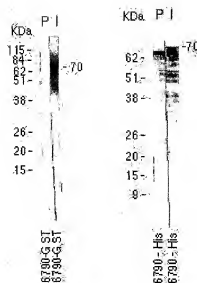
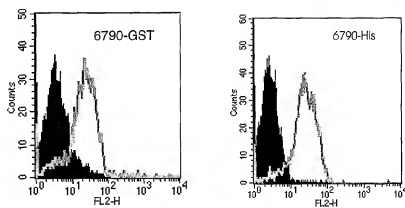
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**FIGURE 50****Fig. 50A****Fig. 50B****Fig. 50C**

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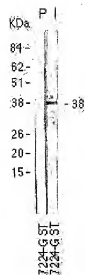
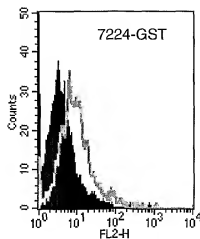
**FIGURE 51****Fig. 51A****Fig. 51B****Fig. 51C**

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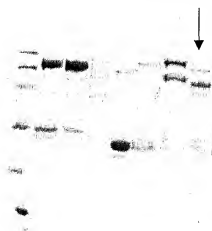
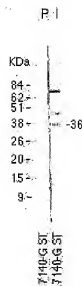
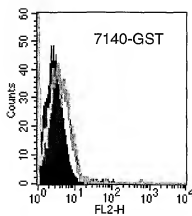
**FIGURE 52****Fig. 52A****Fig. 52B****Fig. 52C**



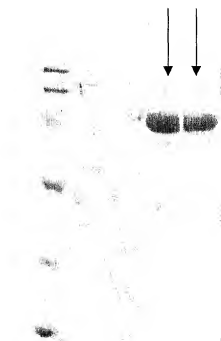
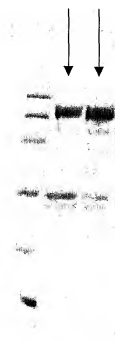
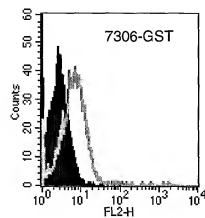
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**FIGURE 54****FIG. 54A****FIG. 54B****FIG. 54C**

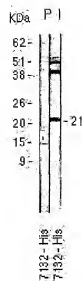
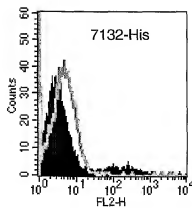
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**FIGURE 55****FIG. 55A****FIG. 55B****FIG. 55C**

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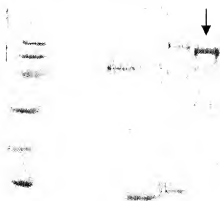
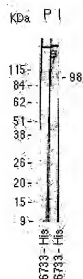
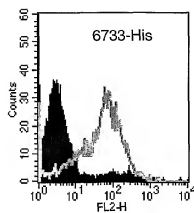
**FIGURE 56****FIG. 56A****FIG. 56B****FIG. 56C****FIG. 56D**

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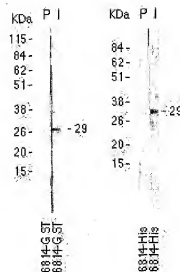
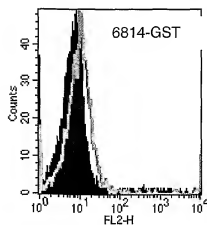
**FIGURE 57****FIG. 57A****FIG. 57B****FIG. 57C**



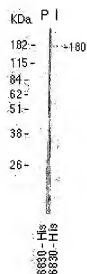
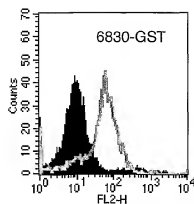
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**FIGURE 58****Fig. 58A****Fig. 58B****Fig. 58C**

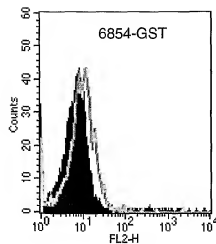
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**FIGURE 59****FIG. 59A****FIG. 59B****FIG. 59C**

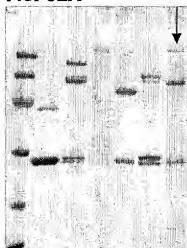
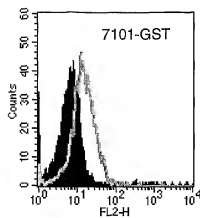
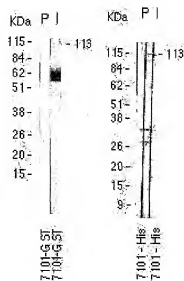
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**FIGURE 60****Fig. 60A****Fig. 60B****Fig. 60C**

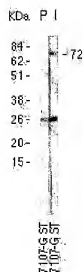
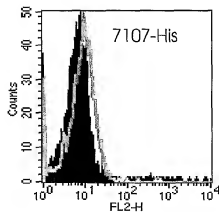
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**FIGURE 61****FIG. 61A****FIG. 61B****FIG. 61C**

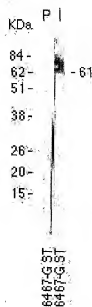
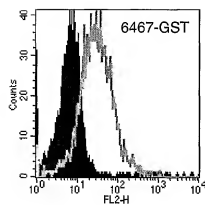
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**FIGURE 62****FIG. 62A****FIG. 62C****FIG. 62B**

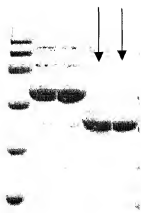
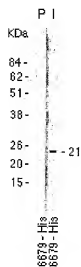
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**FIGURE 63****Fig. 63A****Fig. 63B****Fig. 63C**

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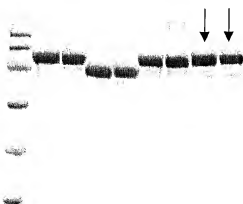
**FIGURE 64****FIG. 64A****FIG. 64B****FIG. 64C****FIG. 64D**

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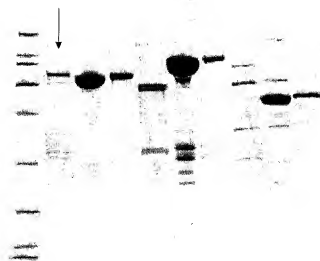
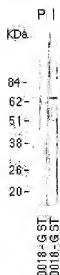
**FIGURE 65****Fig. 65A****Fig. 65B****Fig. 65C**



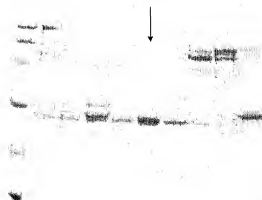
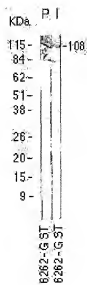
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**FIGURE 66****Fig. 66A****Fig. 66B**

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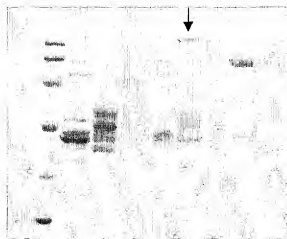
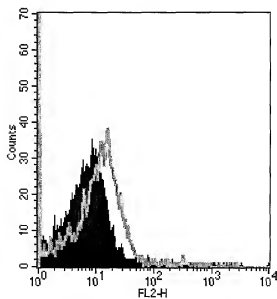
**FIGURE 67****Fig. 67A****Fig. 67B**

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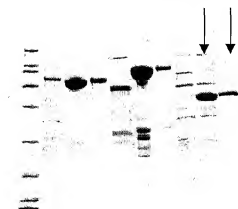
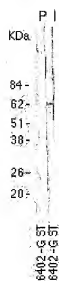
**FIGURE 68****Fig. 68A****Fig. 68B**



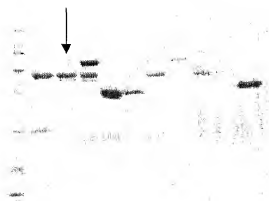
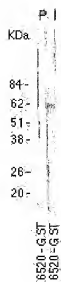
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**FIGURE 70****FIG. 70A****FIG. 70B**

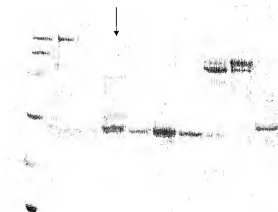
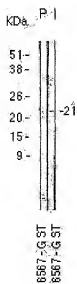
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**FIGURE 71****FIG. 71A****FIG. 71B**

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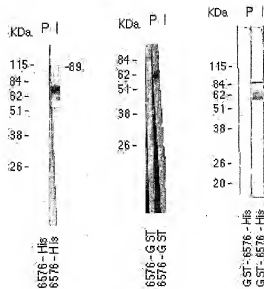
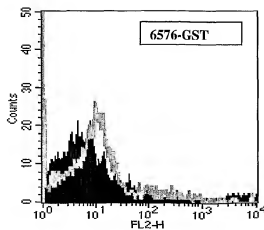
**FIGURE 72****FIG. 72A****FIG. 72B**

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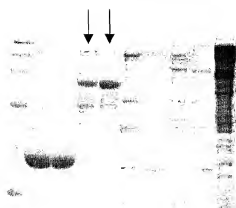
**FIGURE 73****FIG. 73A****FIG. 73B**



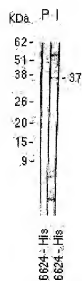
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**FIGURE 74****Fig. 74A****Fig. 74B****Fig. 74C**

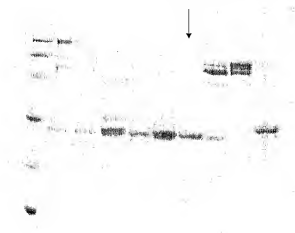
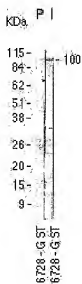
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**FIGURE 75****FIG. 75A****FIG. 75B**

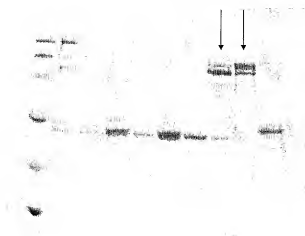
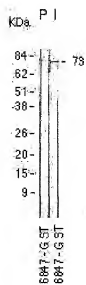
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**FIGURE 76****FIG. 76A****FIG. 76B**

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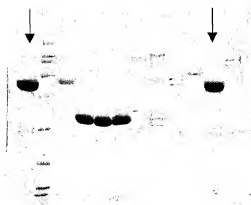
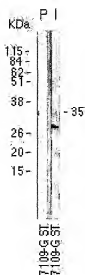
**FIGURE 77****FIG. 77A****FIG. 77B**

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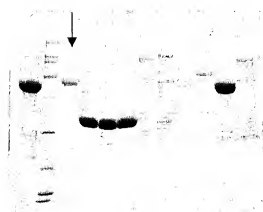
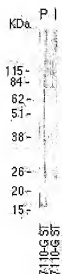
**FIGURE 78****FIG. 78A****FIG. 78B**



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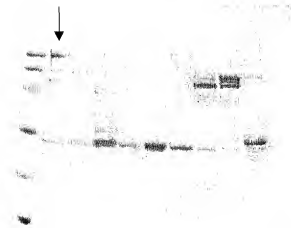
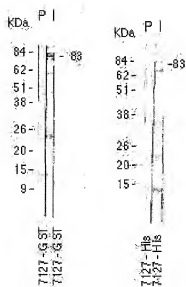
**FIGURE 80****Fig. 80A****Fig. 80B**

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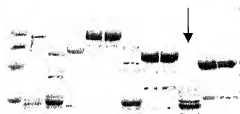
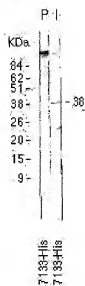
**FIGURE 81****FIG. 81A****FIG. 81B**



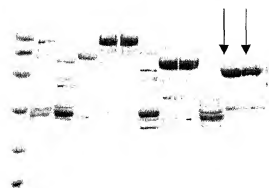
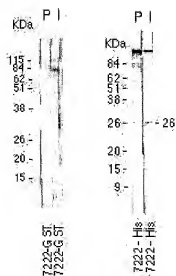
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**FIGURE 82****FIG. 82A****FIG. 82B**

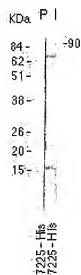
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**FIGURE 83****Fig. 83A****Fig. 83B**

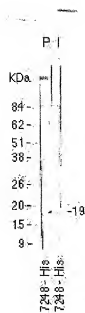
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**FIGURE 84****FIG. 84A****FIG. 84B**

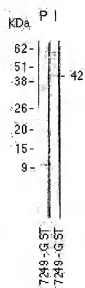
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**FIGURE 85****FIG. 85A****FIG. 85B**

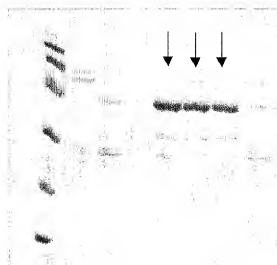
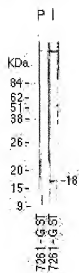
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**FIGURE 86****FIG. 86A****FIG. 86B**

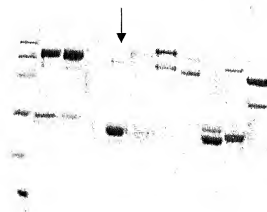
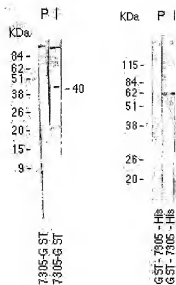
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**FIGURE 87****FIG. 87A****FIG. 87B**

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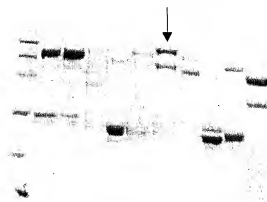
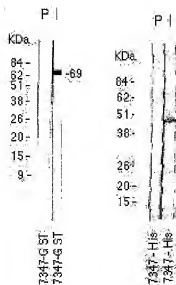
**FIGURE 88****FIG. 88A****FIG. 88B**

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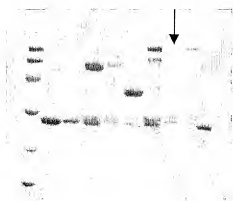
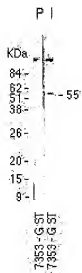
**FIGURE 89****FIG. 89A****FIG. 89B**



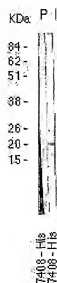
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**FIGURE 90****FIG. 90A****FIG. 90B**

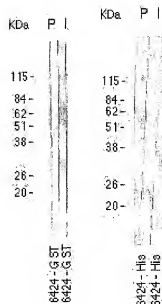
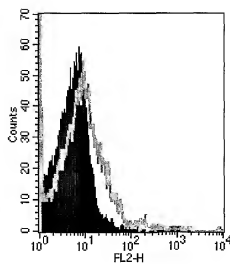
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**FIGURE 91****FIG. 91A****FIG. 91B**

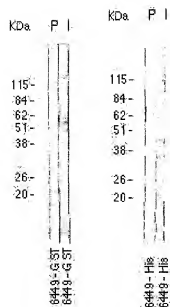
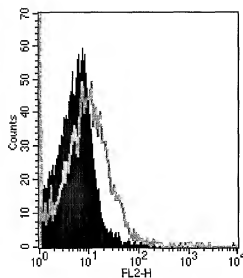
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**FIGURE 92****FIG. 92A****FIG. 92B**

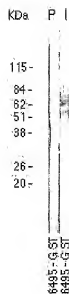
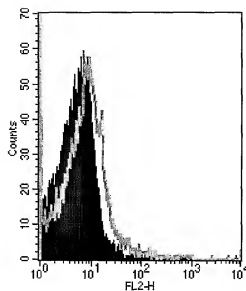
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**FIGURE 93****Fig. 93A****Fig. 93B****Fig. 93C**

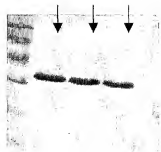
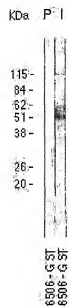
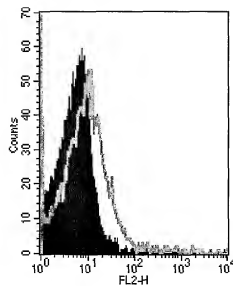
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**FIGURE 94****FIG. 94A****FIG. 94B****FIG. 94C**

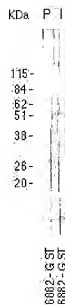
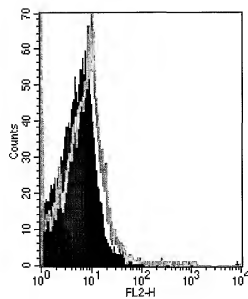
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**FIGURE 95****FIG. 95A****FIG. 95B****FIG. 95C**

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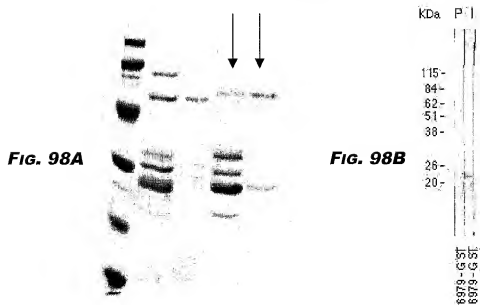
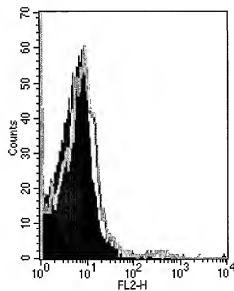
**FIGURE 96****FIG.  
96A****FIG.  
96B****FIG.  
96C****FIG. 96D**

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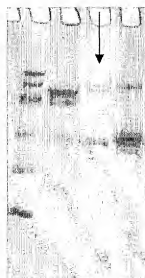
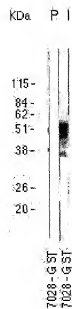
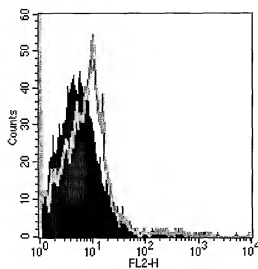
**FIGURE 97****Fig. 97A****Fig. 97B****Fig. 97C**



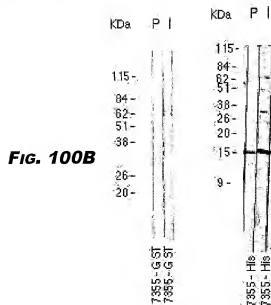
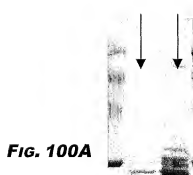
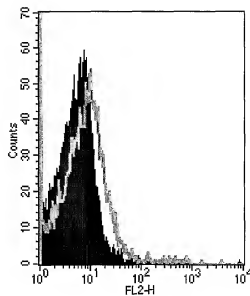
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**FIGURE 98****FIG. 98C**

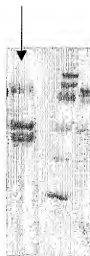
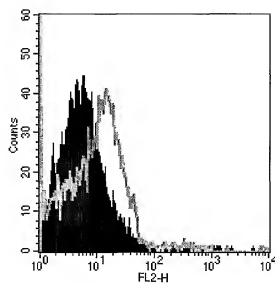
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**FIGURE 99****FIG. 99A****FIG. 99B****FIG. 99C**

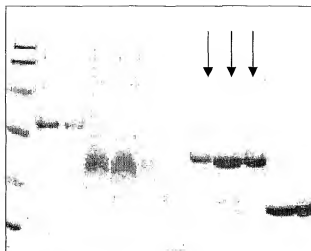
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**FIGURE 100****FIG. 100C**

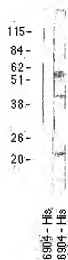
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**FIGURE 101****FIG. 101A****FIG. 101B****FIG. 101C**

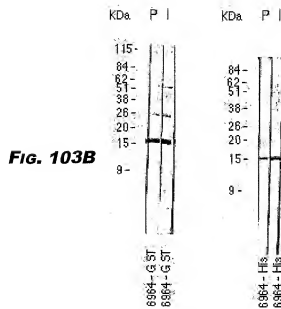
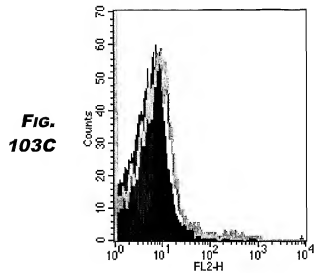
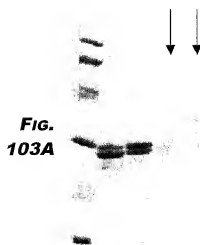
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**FIGURE 102****FIG. 102A**

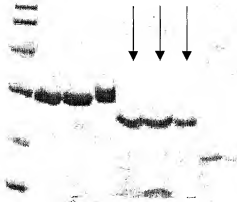
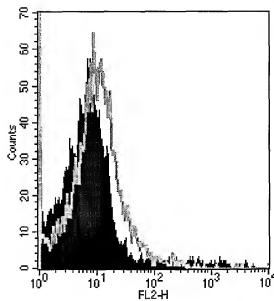
kDa P I

**FIG. 102B**

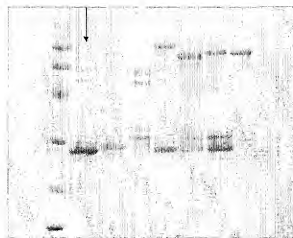
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**FIGURE 103**

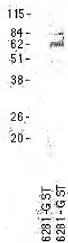
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**FIGURE 104****FIG. 104A****FIG. 104B****FIG. 104C**

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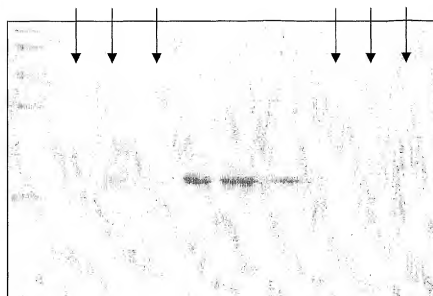
**FIGURE 105****FIG. 105A**

KDa P I

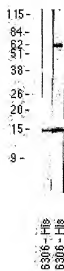
**FIG. 105B**



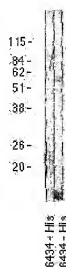
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**FIGURE 106****Fig. 106A****FIG. 106B**

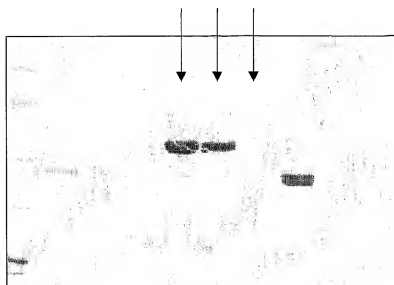
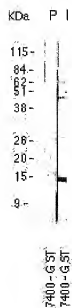
kDa P I

6306 - His  
6306 - His**FIGURE 107**

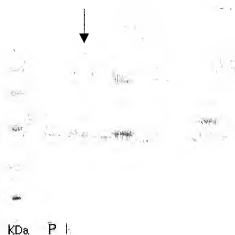
kDa P I

6434 - His  
6434 - His

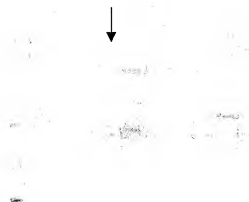
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**FIGURE 108****FIG. 108A****FIG. 108B**

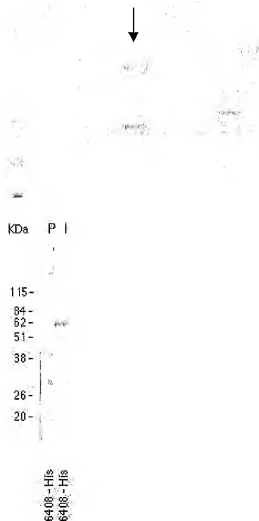
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**FIGURE 109****Fig. 109A****Fig. 109B**

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**FIGURE 110****FIG. 110A****FIG. 110B**

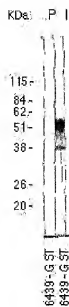
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**FIGURE 111****FIG. 111A****FIG. 111B**

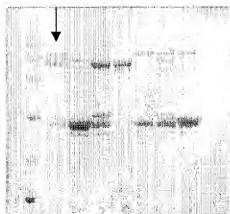
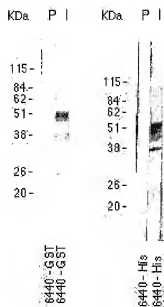
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**FIGURE 112****FIG. 112A****FIG. 112B**

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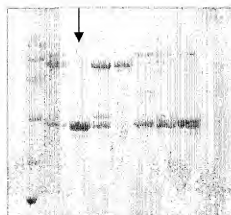
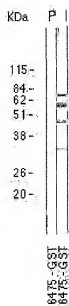
**FIGURE 113****FIG. 113A****FIG. 113B**

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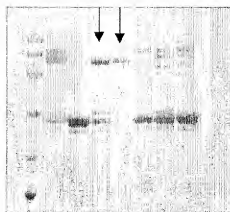
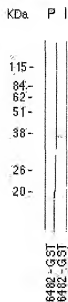
**FIGURE 114****FIG. 114A****FIG. 114B**



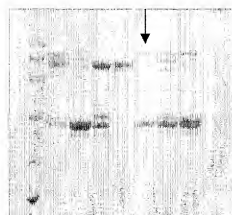
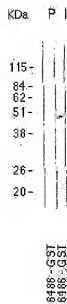
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**FIGURE 115****Fig. 115A****Fig. 115B**

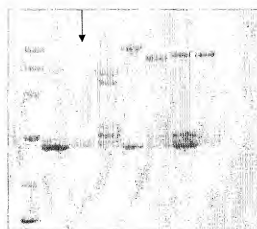
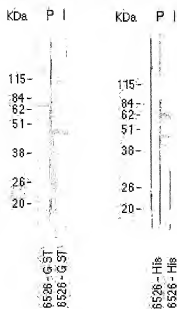
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**FIGURE 116****FIG. 116A****FIG. 116B**

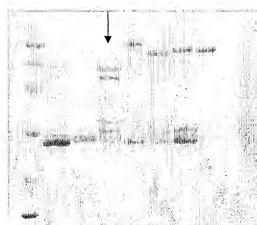
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**FIGURE 117****FIG. 117A****FIG. 117B**

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**FIGURE 118****FIG. 118A****FIG. 118B**

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**FIGURE 119****FIG. 119A**

kDa P I.

115 -

84 -

62 -

51 -

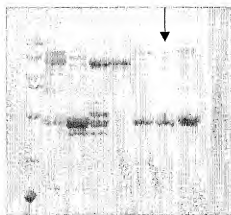
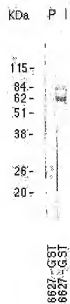
38 -

26 -

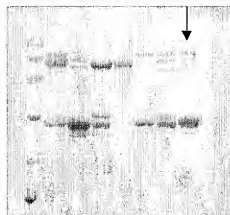
20 -

6528 - G ST  
6528 - G ST**FIG. 119B**

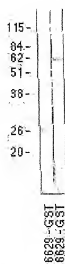
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**FIGURE 120****FIG. 120A****FIG. 120B**

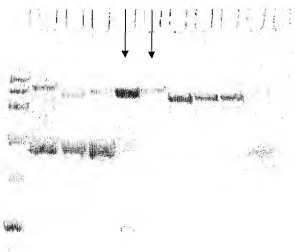
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**FIGURE 121****FIG. 121A**

KDa P I

**FIG. 121B**

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**FIGURE 122****Fig. 122A**

KDa P I

115-

84-

62-

51-

38-

26-

20-

151

135

119

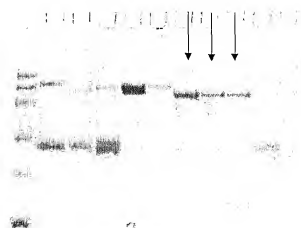
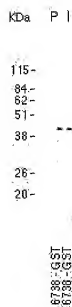
103

87

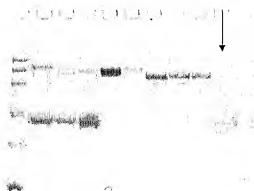
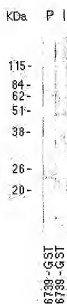
**Fig. 122B**



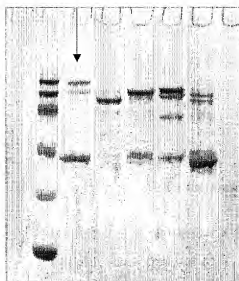
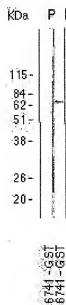
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**FIGURE 123****FIG. 123A****FIG. 123B**

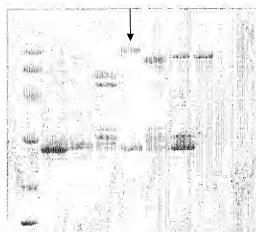
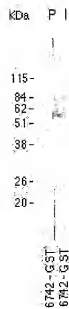
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**FIGURE 124****FIG. 124A****FIG. 124B**

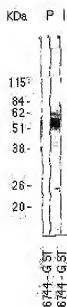
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**FIGURE 125****FIG. 125A****FIG. 125B**

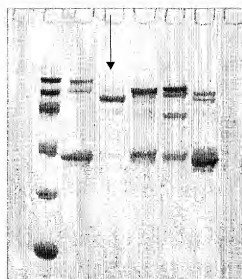
125/169

**FIGURE 126****FIG. 126A****FIG. 126B**

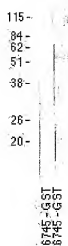
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**FIGURE 127****FIG. 127A****FIG. 127B**

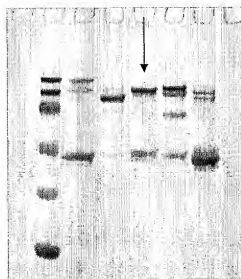
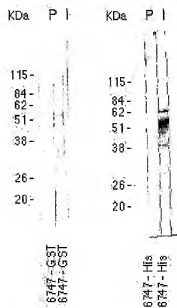
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**FIGURE 128****FIG. 128A**

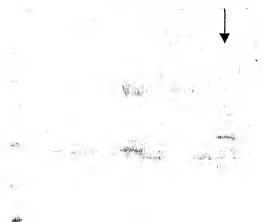
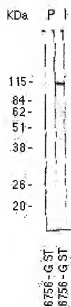
KDa P I

**FIG. 128B**

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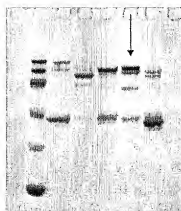
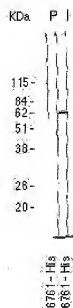
**FIGURE 129****Fig. 129A****Fig. 129B**

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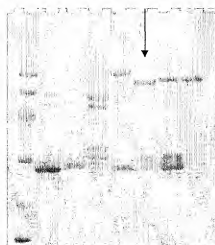
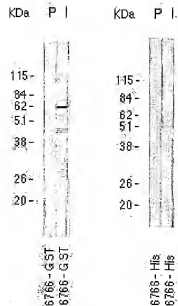
**FIGURE 130****FIG. 130A****FIG. 130B**



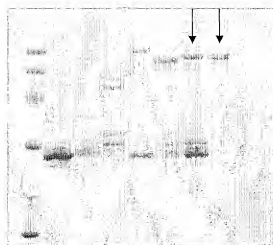
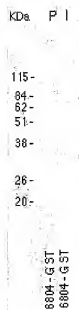
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**FIGURE 131****FIG. 131A****FIG. 131B**

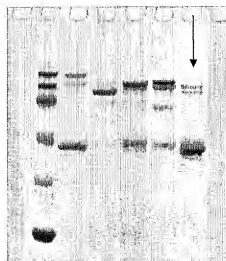
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**FIGURE 132****Fig. 132A****Fig. 132B**

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**FIGURE 133****Fig. 133A****Fig. 133B**

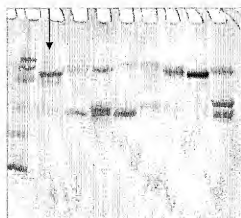
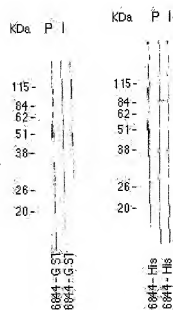
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**FIGURE 134****FIG. 134A****FIG. 134B**

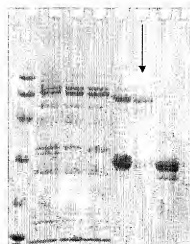
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**FIGURE 135****Fig. 135A****Fig. 135B**

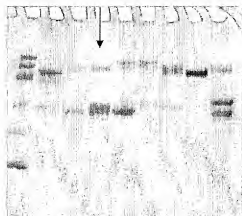
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**FIGURE 136****FIG. 136A****FIG. 136B**

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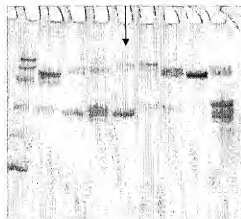
**FIGURE 137****Fig. 137A****Fig. 137B**

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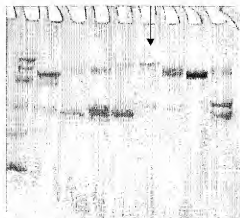
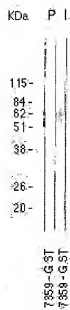
**FIGURE 138****Fig. 138A****Fig. 138B**



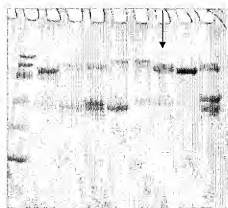
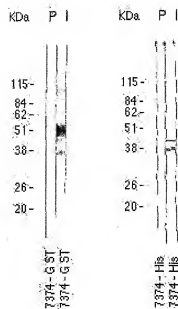
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**FIGURE 139****FIG. 139A****FIG. 139B**

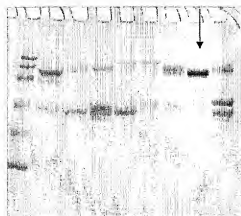
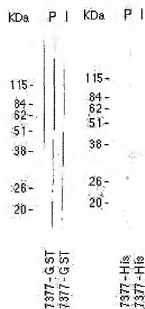
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**FIGURE 140****FIG. 140A****FIG. 140B**

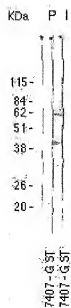
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**FIGURE 141****Fig. 141A****Fig. 141B**

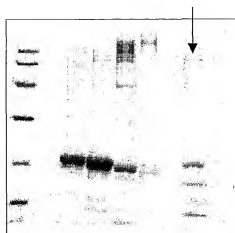
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**FIGURE 142****FIG. 142A****FIG. 142B**

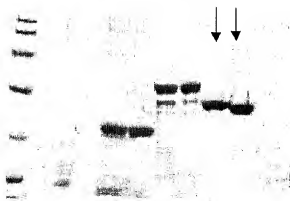
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**FIGURE 143****FIG. 143A****FIG. 143B**

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**FIGURE 144****FIG. 144A****FIG. 144B**

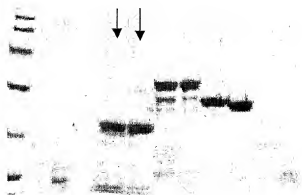
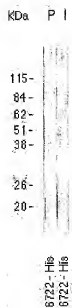
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**FIGURE 145****FIG. 145A****FIG. 145B**

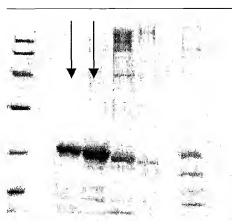
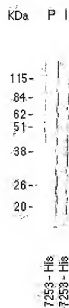




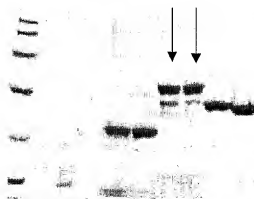
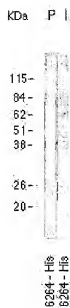
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**FIGURE 147****FIG. 147A****FIG. 147B**

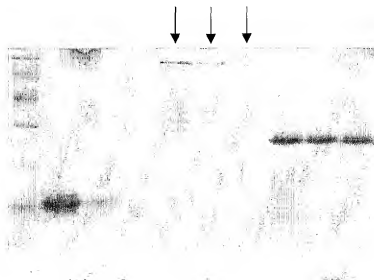
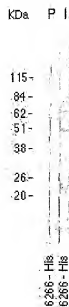
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**FIGURE 148****FIG. 148A****FIG. 148B**

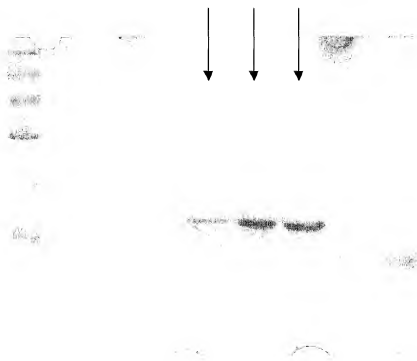
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**FIGURE 149****Fig. 149A****FIG. 149B**

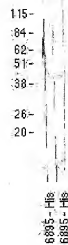
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**FIGURE 150****Fig. 150A****Fig. 150B**

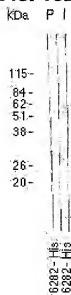
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**FIGURE 151****Fig. 151A****Fig. 151B**

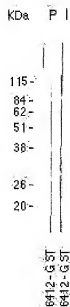
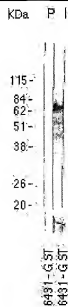
KDa P I



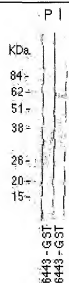
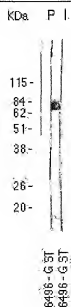
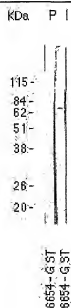
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**FIGURE 152****Fig. 152A****Fig. 152B****FIGURE 153**

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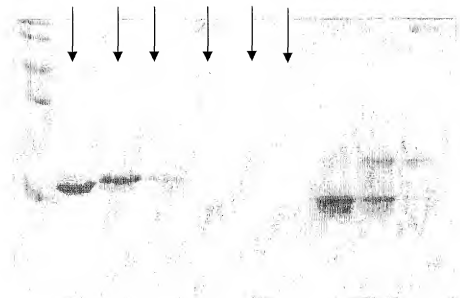
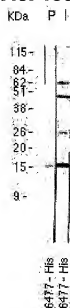
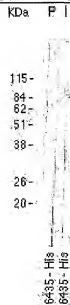
**FIGURE 154****FIG. 154A****Fig. 154B****FIGURE 155**

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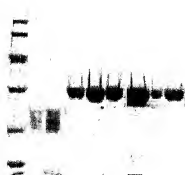
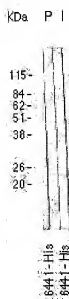
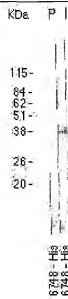
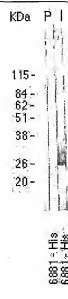
**FIGURE 156****FIGURE 157****FIGURE 158**



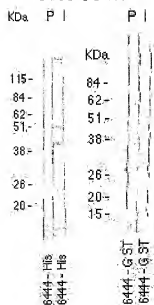
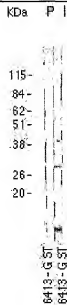
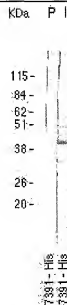
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**FIGURE 159****Fig. 159A****Fig. 159B****FIGURE 160**

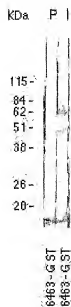
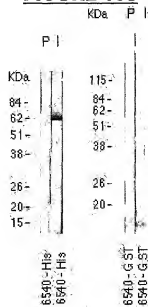
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**FIGURE 161****Fig. 161A****Fig. 161B****FIGURE 162****FIGURE 163**

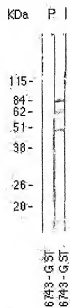
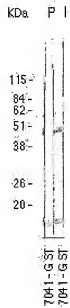
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**FIGURE 164****Fig. 164A****Fig. 164B****FIGURE 165****FIGURE 166**

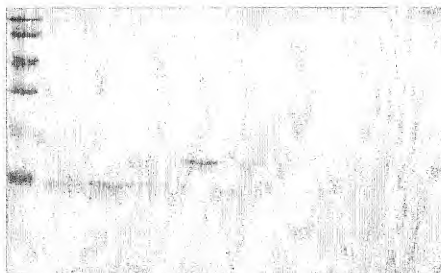
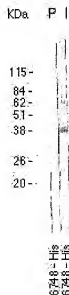
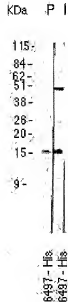
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**FIGURE 167****Fig. 167A****Fig. 167B****FIGURE 168**

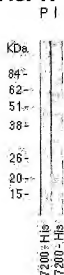
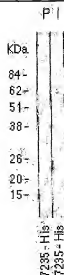
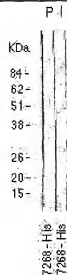
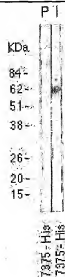
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**FIGURE 169****FIGURE 170**

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**FIGURE 171****Fig. 171A****Fig. 171B****FIGURE 172****FIGURE 173**

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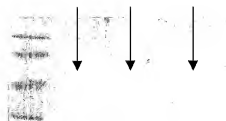
**FIGURE 174****Fig. 174A****FIG. 174B****FIGURE 175****FIGURE 176****FIGURE 177****FIGURE 178**

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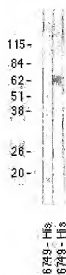
**FIGURE 179****FIG. 179A****FIG. 179B**



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**FIGURE 180****FIG. 180A**

kDa P I

**FIG. 180B**

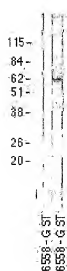
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**FIGURE 181**

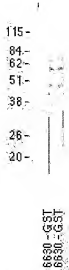
KDa P I

**FIGURE 182**

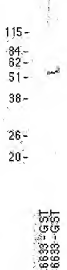
KDa P I

**FIGURE 183**

KDa P I

**FIGURE 184**

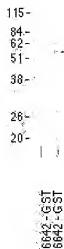
KDa P I



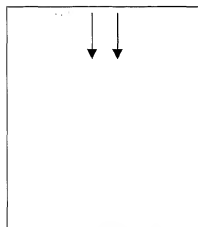
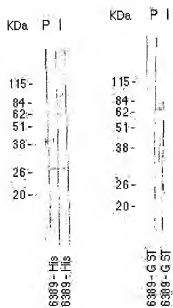
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**FIGURE 185**

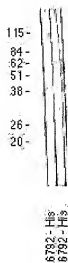
KDa P I



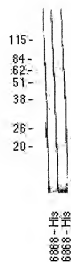
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**FIGURE 186****FIG. 186A****FIG. 186B**

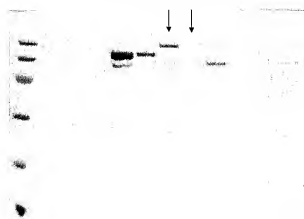
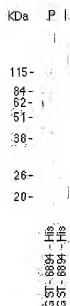
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**FIGURE 187****FIG. 187A****FIG. 187B**

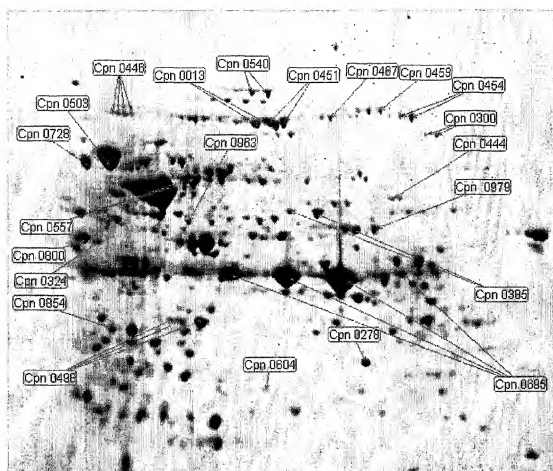
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**FIGURE 188****Fig. 188A****Fig. 188B**

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**FIGURE 189****FIG. 189A****FIG. 189B**

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**FIGURE 190****FIGURE 191**

S V I V G . V S T N S E H R Y H A F Q Y A D G Q M V D L G T L G G P E S Y A Q G V S G D G K  
 K V I V G . H S T R T D G E Y R A F K Y V D G R M I D L G T L G G S A S F A F G V S D D G K  
 K V I V G . R S E T Y Y G E V H A F C H K N G V M S D L G T L G G S Y S A A K G V S A T G K  
 K V I V G . W S T T N N G E T H A F M H K D E T M H D L G T L G G G F S V A T G V S A D G R  
 T I I V G S M E S T I T R K T T A V K W V N N V P T Y L G T L G G D A S T G L Y I S G D G T

